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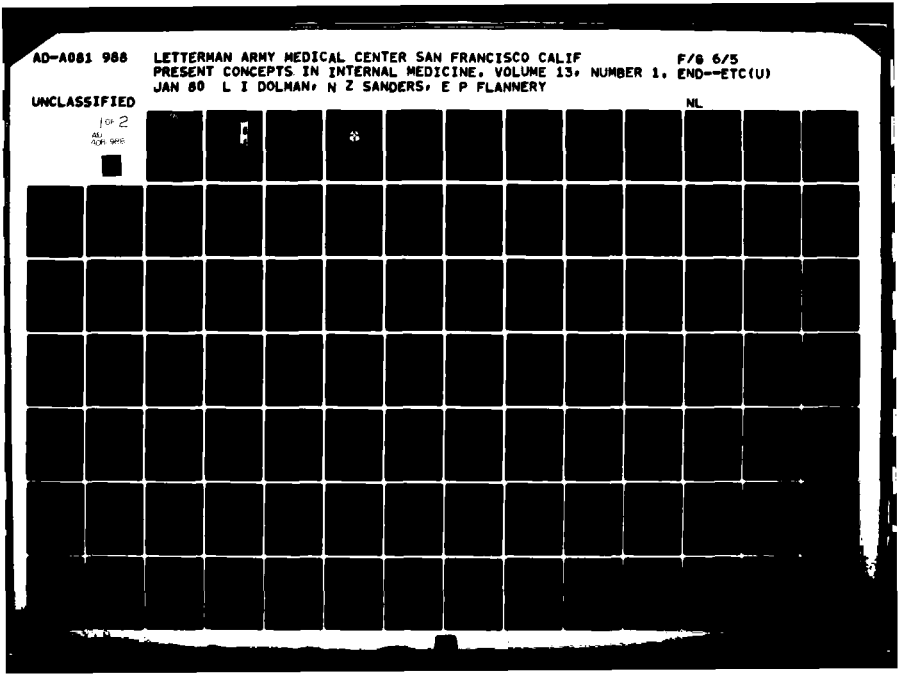

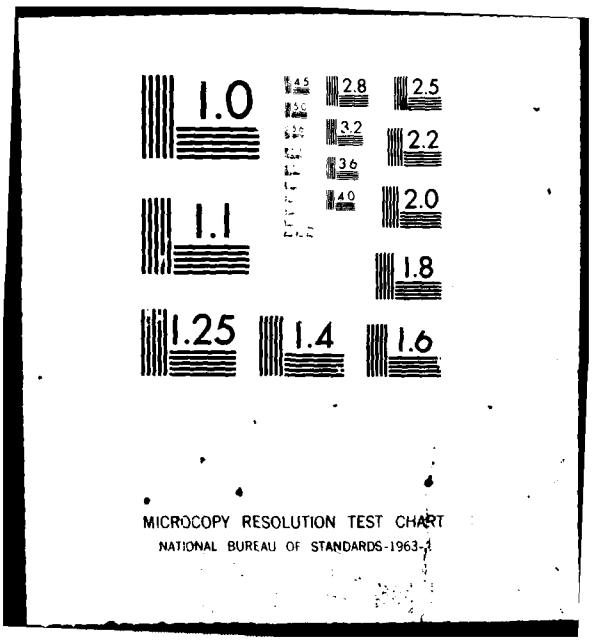
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Letterman Army Medical Center
Presidio of San Francisco, CA 94129

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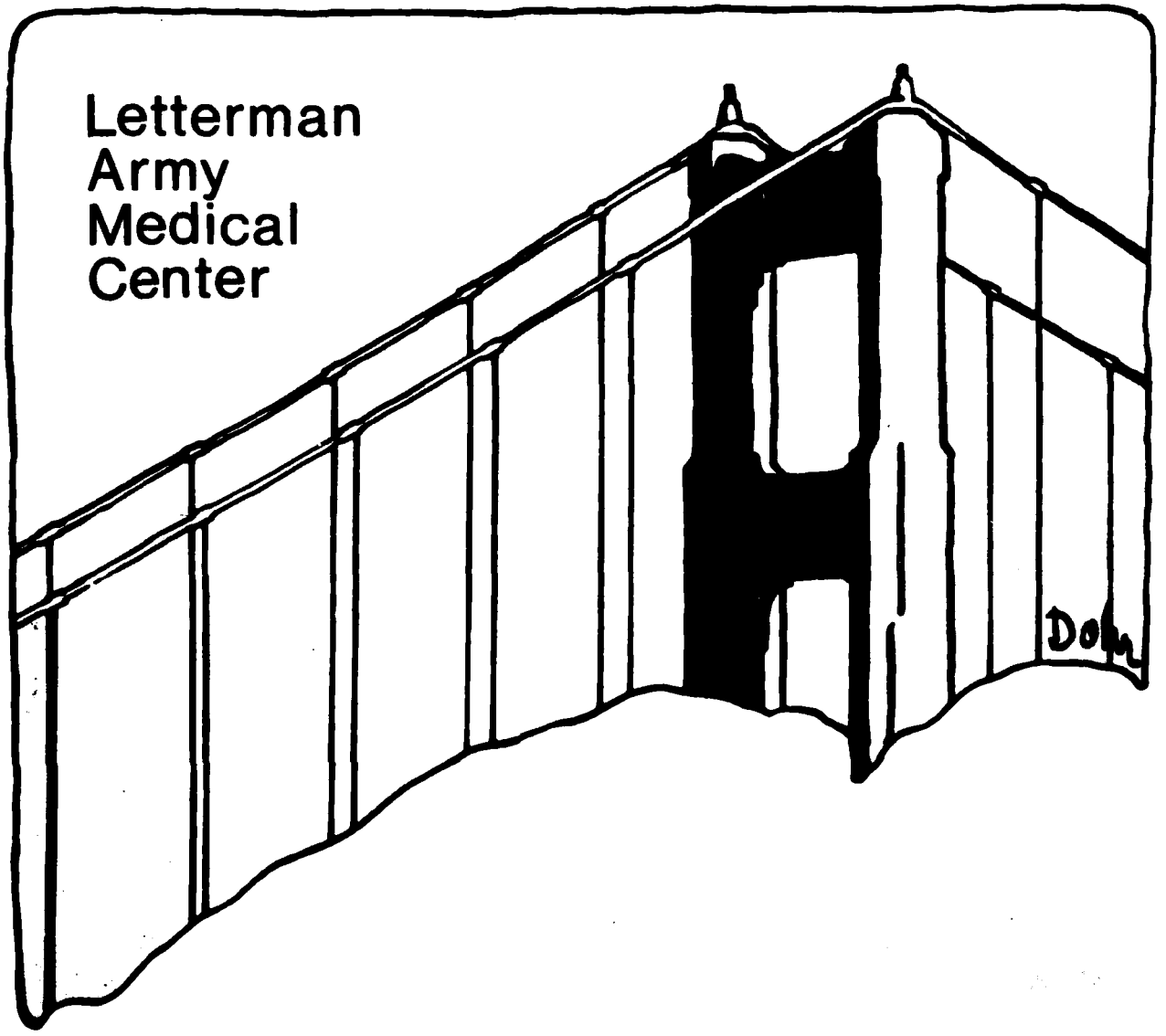

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
This symposium consists of eight articles, a panel discussion, 26 tables, and 13 figures (for quick reference). It includes articles on Cushing's disease diagnosis and treatment of acromegaly; diagnosis and followup of secretory pituitary adenomata; vitamin D; somatomedin and other growth factors; use of the computer in the management and study of diabetes mellitus; studies of the trophic properties of gastrin on the gut; and gastrin in the human small bowel. It also includes a pituitary panel discussion.

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PRESENT CONCEPTS

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Volume 13 Number 1 -
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Loren I. Dolman, ~~MD, FACP~~
Major, Medical Corps, *Editor*

Nina Z. Sanders, BA
Technical Publications Editor

Eugene P. Flannery, MD, FACP
Colonel, Medical Corps
Chief, Department of Medicine

Floyd W. Baker, MD
Brigadier General, Medical Corps
Commander

LETTERMAN ARMY MEDICAL CENTER
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PRESENT CONCEPTS IN INTERNAL MEDICINE

Endocrinology Research Symposium, January 1980

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FOREWARD

Letterman Army Medical Center, located in San Francisco, California, is a major referral center for U.S. military personnel. In addition, it is a training ground for fellows, residents, interns, and medical students. Its staff is actively engaged in both basic science and clinical research. Many of the latter functions are accomplished in association with nearby military and civilian institutions.

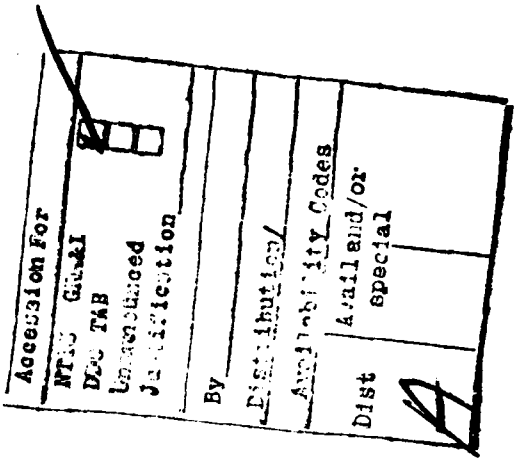
I am pleased to gather for this Symposium authors from Letterman and its associated military and civilian institutions who are presenting some of their work in the field of Endocrinology.

The Panel on Pituitary Disease was held on January 23, 1979, in association with the course, "Present Concepts in Internal Medicine," co-sponsored by the American College of Physicians and Letterman Army Medical Center. I am most grateful to our guest authors, Dr. Wiedemann of the University of California, Berkeley, and Dr. Tyrrell from the University of California, San Francisco, as well as to Nichols Institute for making the Pituitary Panel possible.

The other papers were produced by physicians on active duty with the U.S. Army and Navy working at Letterman Army Medical Center and Letterman Army Institute of Research in San Francisco, and the Naval Regional Medical Center in Oakland. I appreciate their contributions as well.

It is my desire that such collaborative efforts as this Symposium will serve to further solidify the medical community in the San Francisco Bay Area, military and civilian alike.

LOREN I. DOLMAN, MD, FACP
Editor



CUSHING'S DISEASE

*J. Blake Tyrrell, MD

INTRODUCTION

In this discussion I will review our procedures for establishing the diagnosis of Cushing's disease (pituitary ACTH hypersecretion) and the methods of differentiating Cushing's disease from other types of spontaneously occurring Cushing's syndrome (adrenal tumors and the ectopic ACTH syndrome). I will then review our current experience with transsphenoidal pituitary microsurgery in the treatment of Cushing's disease.

CLINICAL PRESENTATION

Cushing's disease typically presents with the manifestations of hypercortisolism and adrenal androgen excess, which, of course, include obesity with predominantly central fat distribution, plethora, hypertension, hyperglycemia or glucose intolerance, hirsutism, muscle weakness, striae, easy bruisability and psychological disturbances. Menstrual disorders or impotence occur in the majority of patients. Other manifestations include acne, osteoporosis, edema and poor wound healing.

Clinical features which suggest the ectopic ACTH syndrome include male sex, rapid onset, weight loss, anemia, pigmentation, hypokalemia, marked steroid hypersecretion and markedly elevated plasma ACTH levels. Patients with adrenal carcinoma also tend to have rapidly progressive and severe Cushing's syndrome with markedly elevated steroid levels which are often predominantly of the ketosteroid fractions. In these patients plasma ACTH levels are suppressed.

*From Metabolic Research Unit and Department of Medicine, University of California, San Francisco, CA

DIAGNOSIS OF HYPERCORTISOLISM

When the clinical suspicion of Cushing's syndrome has been raised, it is our practice to first perform the overnight dexamethasone suppression test on an outpatient basis. Patients with Cushing's syndrome of any etiology have disordered regulation of steroid feedback at the hypothalamic-pituitary level, and this simple and reliable screening test will establish the diagnosis in over 95% of cases.[1,2] False negatives may occur in patients with intermittent or episodic Cushing's syndrome. False positives occur in acute illness, severe depression, high estrogen states (pregnancy, estrogen or oral contraceptive therapy), ethanol excess (alcoholic pseudo-Cushing's) and with chronic diphenylhydantoin or barbiturate therapy (accelerated metabolism of dexamethasone). This rapid overnight test provides the same information as the classic Liddle suppression test in which dexamethasone 0.5 mg q6h (2 mg/day) is given for two days with collection of urine 17-OHCS.[3]

Confirmation of the diagnosis is best established by measurement of urine free cortisol which has the advantage of not being elevated in obesity.[4] False negatives occur in 10% of patients with Cushing's syndrome, particularly those with mild or intermittent hypercortisolism. False positives occur in acute illness, alcoholism and with severe anxiety and/or depression.

Since no single test is entirely reliable in diagnosis, multiple criteria should be used. In our experience (in the absence of conditions causing false positive responses) the presence of elevated urine free cortisol (> 100 µg/24 h) and lack of suppression with overnight dexamethasone establish a diagnosis of Cushing's syndrome. In patients with equivocal results, repeated evaluation may be required before the diagnosis of Cushing's syndrome can be either established or excluded. Traditionally used tests such as urine 17-OHCS, 17-KGS, 17-KS and diurnal plasma corticosteroids give a high incidence of both false positives and false negatives and are much less useful in diagnosis.[2]

DIFFERENTIAL DIAGNOSIS

When Cushing's syndrome (hypercortisolism) has been proven, the specific etiology must be defined, i.e., pituitary ACTH hypersecretion (Cushing's disease) must be differentiated from the ectopic ACTH syndrome and from primary adrenal tumors.

It is our practice to again utilize two procedures to establish the specific type of Cushing's syndrome: the measurement of basal levels of plasma ACTH,[5] and the performance of the high-dose dexamethasone suppression test of Liddle.[3] We are currently evaluating a rapid overnight high-dose suppression test which appears reliable and will shorten the evaluation time of these patients.[6]

Patients with Cushing's disease have "normal" to modestly elevated plasma ACTH levels, with measurable levels in the presence of hypercortisolism being consistent with ACTH-induced bilateral adrenocortical hyperplasia.[5,7] These patients typically have plasma ACTH levels of 50 to 200 pg/ml. Lower levels should raise the suspicion of the presence of an adrenal tumor, and levels above 200 pg/ml suggest the ectopic ACTH syndrome. Patients with Cushing's disease also characteristically maintain some feedback control of ACTH secretion, i.e., there is suppression of urine 17-hydroxycorticoids to less than 50% of basal levels following administration of high-dose dexamethasone. Suppression with dexamethasone is not helpful in determining which patients with Cushing's disease have pituitary tumors, since patients with microadenomas characteristically show suppressibility.[7]

Plasma ACTH levels are usually markedly elevated in the ectopic ACTH syndrome although there is overlap with the values seen in Cushing's disease.[5] Since hypothalamic-pituitary control of ACTH secretion is absent, no suppression with high-dose dexamethasone can be achieved. In addition, the primary tumor is clinically obvious in virtually all patients.

Adrenal tumors function autonomously and suppress the normal hypothalamic-pituitary axis, leading to the characteristic findings of subnormal (< 20 pg/ml) or unmeasurable levels of plasma ACTH and the absence of steroid suppression by high-dose dexamethasone. Adrenal adenomas frequently (60% to 70%) respond to exogenous ACTH stimulation testing, whereas carcinomas do not. As stated above, adrenal carcinomas usually have markedly elevated steroid levels with adrenal androgens predominating (17-ketosteroids),[8,9] whereas adrenal adenomas usually secrete predominantly glucocorticoids with normal or subnormal levels of urine 17-ketosteroids. Since adrenal tumors causing Cushing's syndrome are usually greater than 2 cm in diameter, they can be readily localized by computed tomographic scans and ultrasonography[10,11] or by isotope scans using I-131-19-iodocholesterol,[12] and invasive procedures such as arteriography or venography are rarely necessary.

Adrenal tumors rarely cause diagnostic problems and the diagnosis of the ectopic ACTH syndrome is usually obvious; however, considerable difficulties exist in differentiating pituitary from ectopic ACTH sources when either the pituitary or ectopic tumor is occult. This problem is especially important when contemplating therapy directed to the pituitary in Cushing's disease, since only one-third of patients have definitely abnormal sellar tomograms.[13]

Thus, the major problem in differential diagnosis is separating patients with Cushing's disease from patients with ectopic-ACTH secretion in whom the neoplasm is not clinically apparent. The clinical picture in these patients may be identical to that characteristically seen in Cushing's disease, and steroid levels are only moderately elevated in contrast to the usual case of ectopic ACTH syndrome. A surprising number of relatively benign ectopic tumors have ACTH and steroid dynamics identical to those seen in Cushing's disease, i.e., these tumors (usually bronchial adenomas or thymomas) are dexamethasone-suppressible and show stimulation with metyrapone.[14,15] In addition, CRF-like bioactivity has been demonstrated in a high proportion of tumors producing the ectopic ACTH syndrome, although the clinical significance of these findings is unclear.[16]

Although glucocorticoid suppressibility has been used as a classic index of Cushing's disease, approximately 10% of patients with pituitary ACTH hypersecretion fail to suppress with high-dose dexamethasone, causing further problems in the differential diagnosis of pituitary and ectopic ACTH secretion.[14] Patients with nodular adrenal hyperplasia also usually fail to suppress with usual doses of dexamethasone.[17]

Certain additional diagnostic maneuvers may help to further define those difficult cases in which an occult ectopic lesion is suspected. Sellar tomograms, if abnormal, will help confirm a pituitary etiology; adrenal CT scanning may show nodular adrenal hyperplasia; and higher doses of dexamethasone (16 or 32 mg/day) may cause ACTH suppression in pituitary Cushing's disease.[18,19] In patients with a suspected occult ectopic tumor, careful evaluation of chest radiographs and tomography of the lungs and mediastinum may localize the lesion. In these patients, venous catheterization with ACTH and LPH sampling will frequently differentiate a pituitary from an ectopic source of ACTH and may localize the occult tumor.[20,21] Since ectopic tumors frequently secrete ACTH, LPH, big ACTH, and a variety of biologically inactive ACTH and LPH fragments, the future use of immunoassays with differential sensitivity may be helpful in selected patients if other tests are unsuccessful.

In patients with typical steroid dynamics of Cushing's disease, a pituitary source of ACTH secretion is most likely; however, in those patients with normal sellar polytomography,[13] an occult ectopic source of ACTH cannot be excluded with absolute certainty in any given case. The diagnostic studies described will differentiate most patients, although a minority will continue to be diagnostic and therapeutic enigmas.

PATHOLOGY AND PATHOGENESIS OF CUSHING'S DISEASE

Cushing clearly described the clinical manifestations of hypercortisolism and attributed them to the pituitary microadenomas found at autopsy in his patients.[22] In spite of this clear description of the clinical and pathological features of Cushing's disease, the etiology and pathogenesis

of this disorder has remained controversial. Although a hypothalamic etiology has been proposed,[23,24] both past and current series have shown the presence of pituitary adenomas in the great majority of patients,[7,22,25-30] and recent experience has confirmed that selective surgical resection of these adenomas corrects hypercortisolism.[7,28,29,31]

The characteristic endocrine features of Cushing's disease are well known and include abnormalities of the three basic mechanisms of ACTH and cortisol regulation: 1) there is lack of circadian periodicity; 2) feedback regulation by circulating glucocorticoids is abnormal; 3) there is lack of normal ACTH and cortisol responsiveness to the stress of hypoglycemia and surgery.[5,32]

The endocrine responses to selective transsphenoidal microsurgery have been instructive in elucidating the role of microadenomas in Cushing's disease. As we reported, basal levels of plasma ACTH and cortisol fall rapidly to normal and usually to subnormal levels in the immediate postoperative period.[7,33] With subsequent followup (6-18 months), these levels gradually increase into the normal range and, as expected, plasma ACTH levels return to normal before the plasma cortisol levels. In addition, these patients have maintained normal suppressibility of plasma cortisol levels to low-dose dexamethasone throughout the followup period. The fall in basal plasma ACTH and plasma cortisol was accompanied by hyporesponsiveness of plasma cortisol to exogenous ACTH stimulation (Cortrosyn® 0.25 mg IV). Plasma cortisol was hyperresponsive to ACTH stimulation prior to surgery, became definitely subnormal (normal peak cortisol greater than 20 µg/dl) when studied at two to six months postoperatively, and, with subsequent followup, plasma cortisol responses to ACTH returned to normal. We have also followed plasma ACTH and cortisol responses to hypoglycemia. Preoperatively, plasma ACTH and cortisol showed no increment to insulin hypoglycemia, and at two to six months postoperatively, this relationship was maintained in spite of lower basal levels. In patients followed for more than one year, plasma ACTH responses to hypoglycemia have become normal and the cortisol response has improved, although not yet to normal in every patient. Anterior pituitary function testing in these patients has shown normal growth hormone, TSH, FSH, LH and prolactin responses to appropriate stimuli.

These results indicate that selective adenomectomy removes the sole source of ACTH secretion, and that the para-adenomatous pituitary has suppressed ACTH secretion, resulting in ACTH deficiency and secondary hypo-adrenalism which then gradually recovers following correction of hypercortisolism.

We have shown that selective removal of ACTH-secreting pituitary microadenomas is followed not only by correction of hypercortisolism, but also by transient ACTH deficiency and secondary hypoadrenalism, with subnormal adrenal responsiveness to exogenous ACTH stimulation,[7,33] despite otherwise normal anterior pituitary function. These findings are supportive evidence that the tumor is the sole source of ACTH-secretion and that hypercortisolism results in suppression of the normal hypothalamic-pituitary CRF-ACTH axis which then becomes manifest as 2° hypoadrenalism following tumor removal. Subsequent recovery of normal hypothalamic-pituitary-adrenal function has been demonstrated with normal responsiveness to suppression and stimulation.[31,33,34] We currently believe that, in most cases, Cushing's disease is a primary pituitary disorder due to an ACTH-secreting pituitary microadenoma and that abnormal hypothalamic regulation of ACTH secretion is a consequence of hypercortisolism. ACTH-hypersecretion leads to adrenal hyperplasia with hypercortisolism which, in turn, suppresses hypothalamic CRF secretion. With suppression of CRF the pituitary microadenoma becomes the sole source of ACTH secretion; hypothalamic control of ACTH is absent and therefore hypothalamic-mediated responses such as circadian periodicity and the response to stress and hypoglycemia are abnormal. The characteristic responses to high-dose dexamethasone and metyrapone in this setting of absent hypothalamic control of ACTH secretion are due to direct positive and negative glucocorticoid feedback at the pituitary level, i.e., the tumor itself is responsive to fluctuations in circulating glucocorticoid levels. The evidence that the pituitary itself is a major and perhaps the most important site of glucocorticoid feedback has been reviewed by Lagerquist.[31]

Our interpretation of these results is that a primary ACTH-secreting tumor is the initiating event in most patients with Cushing's disease, and that hypothalamic

abnormalities are reversible following correction of hypercortisolism. Thus, most evidence to date appears to support a primary pituitary etiology of Cushing's disease.

TRANSSPHENOIDAL MICROSURGERY IN CUSHING'S DISEASE

The increasing clinical recognition of ACTH-secreting microadenomas and the clarification of their role in the pathogenesis of Cushing's disease have led to recent advances in the therapy of this disorder. It is our recommendation that primary therapy be directed at the anterior pituitary with the objectives of controlling ACTH hypersecretion, preserving anterior pituitary function and avoiding permanent pituitary or adrenal replacement therapy.

The development of sophisticated microsurgical techniques and increasing surgical experience have now established selective transsphenoidal removal of pituitary microadenomas as an effective primary therapy of Cushing's disease, with a low incidence of morbidity and surgical complications. Recent independent series have confirmed the efficacy of this procedure with a very low incidence of hypopituitarism.[7,28,29]

Our current experience is shown in Table 1. To date, 45 consecutive patients with Cushing's disease have undergone microsurgical exploration of the sella turcica. Preoperative radiologic evaluation showed two distinct groups: 37 patients had microadenomas with normal sellar volume and no extrasellar extension (including 12 who had entirely normal sella polytomograms); and eight patients had sellar enlargement, with suprasellar extension in two, sphenoid extension in five, and lateral tumor extension in one patient. Forty-one of the 45 patients underwent attempted selective tumor removal and four patients required total hypophysectomy.

In the 37 patients with microadenomas, correction of hypercortisolism was achieved in 31 (84%) by selective adenomectomy. The adenomas were 2 to 9 mm in diameter with an average of 5 mm, and approximately 75% were within the body of the normal anterior pituitary. Two patients had persistent hypercortisolism and, in one of these, an adenoma

was not proven despite two surgical explorations. In four patients, no tumor could be identified at surgery and total hypophysectomy was performed with correction of hypercortisolism. Histologic examination revealed small adenomas (1 to 2 mm) in three, and no tumor was identified in the fourth.

TABLE 1

CUSHING'S DISEASE: RESULTS OF PITUITARY MICROSURGERY

	NUMBER	PERCENT
No suprasellar or sphenoid extension present		
– Corrected with selective removal	31	84
– Corrected with total hypophysectomy	4	11
– Persistence or recurrence	2	5
	37	100
Suprasellar or sphenoid extension present		
– Corrected with selective removal	2	25
– Persistence or recurrence	6	75
	8	100

Selective microsurgery, attempted in the eight patients with extrasellar extension, was successful in correcting hypercortisolism in only two. Five patients had persistent hypercortisolism in the immediate postoperative period, and the one patient with lateral extension had initial correction of hypercortisolism with recurrence one year later.

In the entire group of 45 patients, pituitary adenomas were documented in 43 patients (96%) by the criteria of positive histologic findings and/or correction of hypercortisolism with preservation of anterior pituitary function. As stated, in one patient two selective explorations failed to prove an adenoma, and in one no tumor was found despite total hypophysectomy.

Surgical complications in the 41 patients who underwent selective microsurgery were limited in eight (20%) to transient diabetes insipidus which was usually mild, easily managed and which resolved within the first four weeks. Anterior pituitary function was preserved in all of these patients. The four patients undergoing total hypophysectomy have hypopituitarism; three had transient diabetes insipidus and one has permanent diabetes insipidus. One of these patients developed aseptic meningitis but recovered uneventfully.

To date, 23 patients who have had correction of hypercortisolism by selective adenoma removal have been followed for one to five years postoperatively. Of these, seven have been followed for one year, four for two years, and 12 patients for three to five years. Twenty-two patients have continued control of their hypercortisolism, and recurrent Cushing's disease developed in one patient one year following surgery.

Thus, in patients with microadenomas, selective tumor removal was achieved in 84% with rapid and persisting control of hypercortisolism without induction of hypopituitarism. Because of the small size of these tumors, we recommend that surgery be performed by an experienced neurosurgeon who can correctly identify them and preserve pituitary function. Total hypophysectomy may be necessary in some patients and should be reserved for the older patients who have achieved normal growth and puberty and who are not concerned with fertility. Microsurgery is successful in only a minority of patients with extrasellar tumor extension, and multiple therapies, including surgery, radiotherapy and medical treatment, will be necessary in this subgroup of patients with Cushing's disease.

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ACROMEGALY: DIAGNOSIS AND TREATMENT

*Eckehart Wiedemann, MD, FACP

The main purpose of this short review is to discuss some practical aspects of the diagnosis and treatment of acromegaly based in part on some recent data obtained in the large series of acromegalic patients being followed at the Donner Laboratory, University of California, Berkeley.

ETIOLOGY AND PATHOGENESIS

Acromegaly results from chronic growth hormone (GH) oversecretion beginning after epiphysial fusion. Excessive GH secretion beginning during childhood or adolescence causes gigantism which is, however, sometimes associated with classical acromegaly.[1] The pituitary appears to be the exclusive source of the excessive GH. While actual GH secretion by an ectopic source has never been demonstrated, material with GH-like immunoreactivity has repeatedly been extracted from a variety of malignant tumors.[2] Also, the secretion of a substance with growth hormone-releasing activity by a bronchial carcinoid tumor associated with acromegaly has recently been documented.[3] It is still undecided whether the basic abnormality in acromegaly lies in the hypothalamus or in the pituitary itself, or if both a hypothalamic and a pituitary type of acromegaly exist. Most, if not all, of the symptomatology and natural history of acromegaly is attributable to the systemic effects of chronic oversecretion of GH and the local effects of a pituitary tumor. Excessive GH secretion produces overgrowth of skin, bone, cartilage and viscera and thereby gives rise to anatomical manifestations (acral enlargement, degenerative arthropathy, visceromegaly) and biochemical manifestations (e.g., increases of basal metabolic rate, serum phosphorus and urinary calcium and hydroxyproline

*From Donner Laboratory and Donner Pavilion, Lawrence Berkeley Laboratory, University of California, Berkeley, CA.

excretion). GH excess also may cause carbohydrate intolerance due to insulin resistance. This is, however, frequently mitigated by hyperinsulinemia resulting from a direct stimulatory action of growth hormone on the pancreatic islet cells. Increased lipolysis may be responsible for a mild degree of hypertriglyceridemia which is more prevalent in persons with acromegaly than in the population at large.[4] Locally, the tumor produces headaches and may cause visual field defects by compressing the optic nerve or other cranial nerve damage by lateral extension. Frequently, displacement of normal pituitary tissue leads to partial hypopituitarism.

ENDOCRINE ABNORMALITIES

Growth hormone. GH secretion in acromegaly is both quantitatively and qualitatively disturbed: GH secretion is increased and GH secretory dynamics are abnormal.[5,6] Although basal plasma GH levels are almost always elevated, in some cases of unequivocal acromegaly they may be consistently normal on repeated testing. It is important to remember that GH levels are higher in premenopausal women than in men. In adult males, true basal GH concentrations are normally less than 1 ng/ml and rarely greater than 2 ng/ml.[7,8] Thus, in male subjects baseline plasma GH levels consistently greater than 2 ng/ml must raise suspicion. In cases without clearly elevated plasma GH levels, demonstration of abnormal plasma GH responses to stimuli which normally enhance or suppress GH release may establish the diagnosis. In acromegaly, the normal sleep-related rise of GH is absent[9] and arginine administration,[5,6,10] insulin-induced hypoglycemia,[5,6,10] and dopamine agonists (L-dopa, apomorphine, bromocriptine)[10-12] fail to elevate plasma GH concentration and often cause its paradoxical depression.[13-14] Conversely, oral glucose administration does not lower, but often paradoxically elevates, plasma GH levels in acromegaly,[5,6,10,13,14] and injection of thyrotropin-releasing hormone which has no effect on GH levels in normal subjects rather consistently increases plasma GH in acromegaly.[6,15] The TRH test and the oral glucose tolerance test are the most reliable tests of GH secretory dynamics. In the Donner Laboratory series, paradoxical GH

elevations after TRH occurred in 90%, and, after oral glucose administration, in 30% of patients with acromegaly. However, paradoxical GH responses are not diagnostic of acromegaly since they may also occur in patients with chronic renal failure, cirrhosis of the liver, Wilson's disease, Huntington's chorea and acute intermittent porphyria.

Other pituitary hormones. Plasma concentrations of several hormones other than GH are often elevated in acromegaly (Table 1). Of the pituitary hormones, this is particularly true for prolactin. Hyperprolactinemia does not, however, correlate with the presence or absence of galactorrhea in acromegaly. Beta-lipotropin, an anterior pituitary hormone of as yet unknown biological function, is also frequently elevated in acromegaly.[16] On the other hand, about one-half of all acromegalic patients have at least partial deficiencies of one or more pituitary hormones at the time of diagnosis. These deficiencies most commonly affect FSH and LH, whereas clinically significant ACTH deficiency is quite unusual.

TABLE 1

FREQUENCY OF ELEVATED BLOOD HORMONE LEVELS AMONG UNTREATED ACROMEGALICS
(Donner Laboratory, University of California, Berkeley.)

HORMONE	ELEVATED/TOTAL	% ELEVATED
Plasma GH	189/193	98
Serum somatomedin C by RIA	26/26	100
Serum somatomedin by RRA	50/51	98
Serum somatomedin by BA	124/167	74
Plasma prolactin	36/102	35
Plasma B-lipotropin	7/23	30

Abbreviations: BA = bioassay; RIA = radioimmunoassay: RRA = radioreceptor assay.

Somatomedins. Somatomedins are growth hormone-dependent polypeptides produced in the liver which mediate the action of GH on the skeletal tissues. It is, therefore, not surprising that serum somatomedin activity is increased in acromegaly (Table 1). In our experience, elevations of serum somatomedin activity measured by a radioimmunoassay rather specific for somatomedin C,[17] or by a radioreceptor assay which does not distinguish between the various somatomedins,[18] are at least as frequent as elevations of basal plasma GH concentration. Somatomedin activity measured by bioassay is normal in about one-fourth of all acromegalic patients. Nevertheless, measurement of bioassayable somatomedin can be of considerable help in cases with low plasma GH levels, since it is often elevated in such patients (Table 2).

TABLE 2

SERUM SOMATOMEDIN ACTIVITY IN ACROMEGALICS WITH LOW GH

Patient		GH (ng/ml) during OGTT				SM*
Age	Sex	0′	60′	90′	120′	U/ml
37	F	2.3	2.7	2.8	3.9	2.44
62	F	3.0	2.2	1.9	2.5	2.08
45	M	3.4	3.2	2.8	2.9	4.45
53	M	3.8	3.8	3.8	4.0	5.26

* Determined by bioassay; normal: 0.45 - 1.70 u/ml.

Parathyroid hormone. Hyperparathyroidism occurs more frequently in the acromegalic population than in the general population. A small minority of acromegalic patients (less than 2%) have multiple endocrine adenomatosis, type I: pituitary adenoma, primary hyperparathyroidism due to chief-cell hyperplasia, and gastrin-secreting islet cell tumor.

RADIOGRAPHIC FINDINGS

X-ray findings are of considerable help in the diagnosis of acromegaly and are often virtually diagnostic. In more than 90% of cases presently diagnosed, the sella turcica is either enlarged or shows other evidence of abnormality such as asymmetry or erosion. Such irregularities are often revealed by polytomography. Skull films may also show large paranasal sinuses, hyperostosis and prognathism. Tufted phalanges, soft tissue thickening and a sesamoid index[19] increased to over 30 mm^2 are seen on hand films and increased heel pad thickness (males greater than 20, females greater than 18 mm)[20] may be detected on lateral films of the os calcis.

DIAGNOSIS

Presently, in the vast majority of acromegalics the diagnosis is made at an advanced stage of the disease. The mean duration of symptoms in 120 Donner Pavilion patients prior to diagnosis was more than eight years.[8] In contrast to patients with other functioning pituitary tumors, more than 90% of these patients had obvious abnormalities of the sella turcica at the time of diagnosis.[8] Since smaller tumors respond better to therapy, early diagnosis is important. This means that sufficient diagnostic studies should be performed in all patients presenting with such non-specific complaints as headaches, lethargy and weakness, paresthesias and arthralgia, even in the absence of the typical physical stigmata of acral overgrowth and soft tissue thickening. Patients do not always volunteer the most frequent and most characteristic symptom of

acromegaly, hyperhidrosis, and the associated unpleasant body odor. The possibility of acromegaly should also always be considered in the differential diagnosis of carpal tunnel syndrome,[21,22] degenerative joint disease,[21] hypertension,[23,24] and diabetes mellitus, since these disorders are common in acromegaly, occurring in about 70%, 60%, 22%, and 20% of patients, respectively. A suggested work-up schedule for patients suspected of having acromegaly is shown in Table 3.

TABLE 3
WORK-UP FOR ACROMEGALY

A. Screening tests

1. Chemistries (serum P)
2. Plasma GH, fasting and/or 1 hour after oral glucose load
3. X-rays: skull (plain AP + lateral), hands, heel pad

B. To establish unequivocal diagnosis

1. Oral GTT (for GH)
2. TRH test (for GH, TSH, PRL)
3. In borderline cases: serum somatomedin; L-dopa, ITT

C. To assess degree of hypopituitarism

1. Plasma cortisol; T_4, T_3U; testosterone, FSH, LH
2. Metyrapone test for plasma deoxycortisol

D. To assess tumor size and configuration

1. Visual fields
2. Polytomography (2mm cuts) of sella turcica
3. Pneumoencephalography
4. Rarely: arteriogram; cavernous sinus venogram

If any of the screening tests is consistent with the diagnosis (elevated serum phosphorus, elevated fasting or one hour post-glucose plasma growth hormone, suggestive x-ray findings), further work-up is necessary. This usually requires a complete oral glucose tolerance test and/or a TRH test. In equivocal cases, serum somatomedin activity determination and further dynamic tests may be required. Besides establishing the diagnosis, diagnostic studies should determine the degree of hypopituitarism and should assess the size of the tumor and any possible neurological damage caused by it.

THERAPY

Surgical treatment. Among the various surgical procedures employed in the treatment of acromegaly, transsphenoidal microsurgery is clearly the method of choice. It is now widely available and has occasionally resulted in complete cure with restoration of normal basal plasma GH levels and normal GH secretory dynamics without detectable impairment of other pituitary functions.[25] In 75% to 85% of patients, postoperative plasma growth hormone levels were less than 10 ng/ml.[26,27] The incidence of immediate post-surgical hypopituitarism appears to be relatively low. However, there are no long-term followup data, and the frequency of recurrence as well as of hypopituitarism many years after surgery is unknown. Furthermore, although transsphenoidal surgery is a relatively minor procedure, there is associated morbidity, including meningitis, spinal fluid rhinorrhea and diabetes insipidus, as well as mortality.[26,27] Other procedures, such as cryohypophysectomy, stereotaxic thermal coagulation, and implantation of radioactive yttrium or gold have been less successful and are fraught with much greater risk.

Radiotherapy. Three forms of external radiotherapy are presently available, namely, conventional supervoltage irradiation, proton irradiation and alpha particle irradiation.

Conventional supervoltage irradiation is effective in most patients,[28,29] but its effects are very slow. In the

most recently reported large series, five years after irradiation, plasma GH concentration was less than 10 ng/ml in 73% and less than 5 ng/ml in 42% of patients; 10 years after treatment, corresponding figures were 81% and 69%, respectively.[29] Hypoadrenalism was present in 30% after five years and in 38% after 10 years following therapy.[29] Although serious side effects are uncommon, progressive visual failure has occurred in four out of 23 acromegalic patients treated with external megavoltage pituitary irradiation.[30]

Alpha particle pituitary irradiation. Since 1958, more than 300 acromegalic patients have received alpha particle pituitary irradiation from the 184-inch synchrocyclotron at the Lawrence Berkeley Laboratory in Berkeley.[31-34] For this treatment, the patient's head is positioned in an individually fitted plastic holder and is rotated in two planes so that the sella turcica is exposed to the plateau portion of the 910 MeV alpha particle beam which passes through an aperture selected to precisely fit the patient's sella. Treatment consists of four sessions of three to four minutes each, which are completed within one week. The patient is ambulatory, and the procedure is painless and not associated with any mortality or morbidity. The only contraindication is any prior pituitary radiotherapy. However, patients are not accepted for treatment if: a) The optic chiasm cannot be adequately defined by pneumoencephalography; b) there is a suprasellar extension which is not sufficiently separated from the optic chiasm; or c) there is an extension into the sphenoid sinus which cannot be clearly delineated on polytomography. Following alpha particle pituitary irradiation, GH levels usually decrease by more than 50% during the first year and continue to fall more slowly thereafter. After five years, plasma GH levels were less than 10 ng/ml in 82% and 5 ng/ml in 51% of patients; the corresponding values after 10 years were 85% and 78%, respectively.[34] Thus, plasma GH decreases slightly faster than after conventional radiotherapy. On the other hand, the incidence of hypopituitarism is virtually the same with the two modes of therapy.[29-34] No significant neurological complications have been observed since 1961.

Proton beam therapy. Pituitary irradiation with the Bragg peak of a proton beam was originally plagued by

serious side effects which have been eliminated by reduction of the total dose. The results of treatment now appear to be similar to alpha particle therapy.[35]

DRUG THERAPY

Various drugs have been used in the past for treatment of acromegaly, including estrogen, medroxyprogesterone, and chlorpromazine, but proved either ineffective in the great majority of patients or produced unacceptable side effects. The only drug known to be rather regularly effective in reducing plasma growth hormone levels in acromegaly, and in improving the clinical symptoms, is bromocriptine. This drug has been used abroad for long-term treatment of acromegalic patients with considerable success,[36,37] but has not yet received FDA approval for this use. Side effects occur relatively frequently, and include nausea, vomiting and hypotension. Larger doses are required for suppression of growth hormone levels than for reduction of hyperprolactinemia. After cessation of therapy, growth hormone levels increase again and symptoms return. Thus, bromocriptine may be useful in the treatment of certain patients with severe symptomatology who are not amenable to other forms of therapy.

CONCLUSION

Acromegaly at present is usually diagnosed at a far advanced stage. Careful testing of GH secretory dynamics and determination of serum somatomedin activity could lead to an earlier diagnosis in questionable cases. Treatment is more successful when the tumor is small. Best therapeutic results are presently achieved with alpha particle pituitary irradiation and with selective transsphenoidal adenomectomy. Bromocriptine may be useful for temporary control of symptoms, particularly in conjunction with radiotherapy.

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SECRETORY PITUITARY ADENOMATA: DIAGNOSIS AND FOLLOW-UP AFTER SELECTIVE TRANSSPHENOIDAL PITUITARY ADENECTOMY

*Loren I. Dolman, MD, FACP
*Theodore S. Roberts, MD, FACS
*Frank H. Tyler, MD, FACP

Hormone-secreting pituitary adenomata hold a fascination for the physician by presenting clinical manifestations characteristic of the particular hormone or hormones they secrete. The classic signs and symptoms of growth hormone (GH) hypersecretion in acromegaly[1] and ACTH hypersecretion in Cushing's disease[2] are well known. More recently, the syndrome of amenorrhea and galactorrhea resulting from prolactin hypersecretion has been characterized.[3]

Some pituitary adenomata present late in their course, either because they secrete no hormones which produce a characteristic syndrome, or because the hormones have not had recognized symptomatic effects on the patient. These adenomata often become quite large, and cause symptoms by hypothalamic destruction and compression of the optic chiasm.[4] Others may progress slowly in size over decades or actually "burn out."[5]

With the availability of radioimmunoassays for peptide and steroid hormones, the presence of secretory adenomata can now be detected while the lesions are quite small, many not visible on routine x-ray examinations of the sella turcica.[6]

Thus, after the initial clinical impression, the endocrine laboratory remains the basis of diagnosing the pituitary hypersecretory states. In Cushing's syndrome, diagnosis is made by poor suppressibility of plasma cortisol after administration of dexamethasone.[7,8] In

*From University of Utah Medical Center, Salt Lake City, Utah

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acromegaly, one finds persistently elevated GH levels which do not suppress appropriately after a glucose load.[9]

The prolactin-secreting pituitary adenoma has been defined only since the radioimmunoassay for prolactin became available in 1971.[10] Not only has it been demonstrated that at least 30% of pituitary adenomata previously thought to be nonfunctional secrete prolactin, but also that many patients with the syndrome of amenorrhea and galactorrhea,[3] and some males with infertility,[11] have prolactin-secreting pituitary adenomata. The basis of laboratory diagnosis rests in finding persistently elevated prolactin levels,[12] and an abnormal sella turcica on x-ray studies. Although various stimulation and suppression tests have also been utilized, their value is limited.[13]

TSH-secreting pituitary adenomata are much less common. Although some of these adenomata have caused overt hyperthyroidism,[14] most have been reported in hypothyroid patients.[15] Presumably long-standing primary hypothyroidism results in pituitary TSH cell hyperplasia and eventually adenoma formation, as has been shown in animal models.[16] Diagnosis lies in a persistently elevated TSH after adequate thyroxine replacement.

There are also rare cases of LH and FSH hypersecretion,[17,18] caused by a pituitary adenoma.

Unfortunately, the pathological examination of pituitary adenomata has generally not been helpful in determining specific hormone secretion, since most are not well-stained by routine hematoxylin and eosin preparations and are thus labeled "chromophobe" adenomas. There is evidence that many pituitary adenomata can secrete multiple hormones,[19-22] probably from a single cell type as has been suggested by *in vitro* studies.[23-25]

Therapy for pituitary adenomata is controversial. One would not dispute that for larger adenomata that have grown to a size compressing the optic chiasm, or cause pituitary insufficiency, immediate intervention is necessary. Likewise, prolonged hypersecretion of ACTH or GH may cause severe disability and demise of the patient, so that therapy should not be delayed in these situations.[26,27]

With small prolactin-secreting adenomata, neither the size of the adenoma, nor its hormone product is immediately dangerous (although some authors on the basis of animal data have suggested otherwise).[28,29] The major effects are hypogonadism, infertility, and galactorrhea, all compatible with normal life, provided children are not desired. Thus, many physicians would not recommend treatment for prolactin-secreting pituitary adenomata unless immediate fertility were the goal.

Types of treatment for pituitary adenomata are varied. Surgical therapy has always had a role in these syndromes. Initially, the transfrontal surgical approach was utilized, but the procedure has a high morbidity, almost always leaving the patient hypopituitary, and has a significant mortality.[30]

Transsphenoidal pituitary adenectomy, first popularized by Cushing[31] in 1914, was abandoned for many years because of technical problems resulting in high incidence of postoperative meningitis and CSF rhinorrhea. However, in recent years, with certain surgical advances, the procedure has been revived and used in patients with all types of hormone-secreting pituitary adenomata.[32] Bilateral adrenalectomy was a popular treatment for ACTH-overproduction prior to the revival of the transsphenoidal procedure,[33] and is still preferred in some institutions.

Medical therapy has been used in the various hypersecretory pituitary adenomata, and is often directed at the specific hormonal excess. Thus, in Cushing's disease, blockers of adrenal steroid synthesis such as aminoglutethimide[34] and o,p-DDD[35] have been tried. In prolactin-hypersecretion gonadotropins, (LH/FSH)[36] and clomiphene citrate[37] have been used to induce gonadal function.

Recent attempts have been made to directly suppress pituitary hormone overproduction with pharmacologic manipulation. The most widely used drug is the ergot alkaloid derivative, bromocriptine (Parlodel®).[38] Lergotrile mestylate[39] has also been tried. These drugs are effective in reducing prolactin levels to normal in patients with prolactin-secreting pituitary adenomata, allowing return of normal gonadal function, and cessation of

galactorrhea. These drugs have also been used with some success in GH and ACTH overproduction by the pituitary.[40,41] Other drugs such as cyproheptadine, a serotonin antagonist, have had some use in suppressing ACTH overproduction from pituitary adenomata,[42] but there is little evidence that GH or prolactin hypersecretion is effectively suppressed.[43-45]

The problem with all medical therapy for pituitary adenomata is that the adenoma is not cured; the hormonal excess is merely alleviated. Thus, occasional adenomata, especially in the pregnant patient, can grow quite rapidly, causing visual impairment and necessity for immediate surgery.[46]

Radiotherapy has been widely used in acromegaly and Cushing's disease, and is still the mainstay of treatment for these disorders at some institutions.[47-49] There have been few reports on the efficacy of radiotherapy for prolactin-secreting pituitary adenomata.

Finally, in the case of TSH hypersecretion with coincident hypothyroidism, a trial of exogenous thyroid hormone is warranted.[50] If TSH hypersecretion is proven to be autonomous, surgical or radiation therapy may be required.

At the University of Utah Medical Center, we have had the opportunity to examine 63 patients with evidence of pituitary enlargement and hormone overproduction during the last five years. A breakdown of the various hormone types is shown in Table 1.

Fifty-four of the hypersecreting patients eventually underwent transsphenoidal pituitary adenectomy, and the four with TSH hypersecretion were treated with thyroxine replacement. No attempt was made to eliminate the very large adenomata from our series where only reduction of adenoma mass and decompression were the surgical goal. This group includes five patients with prolactin-hypersecretion and nine patients with GH-hypersecretion.

Most of the patients had extensive endocrine evaluation before and after surgery. We present these patients, with a discussion of their diagnosis and evaluation of the use of transsphenoidal pituitary adenectomy in their therapy.

TABLE 1

HORMONE PRODUCTION BY ADENOMATA

PRL alone	29	35%
PRL + GH	3*	4
PRL + ACTH	1	1
PRL + TSH	2	2
PRL + LH	1	1
GH alone	17	21
ACTH alone	8	10
TSH alone	2	2
"Nonfunctional"	19	23
TOTAL	82	

* 15% of GH-secreting, 8% of PRL-secreting

MATERIALS AND METHODS

Between September 1972 and May 1977, 63 patients with evidence of a hormone-secreting pituitary adenoma were evaluated by the University of Utah Division of Endocrinology in collaboration with the Departments of Neurosurgery and Obstetrics-Gynecology. Fifty-four of these patients were treated with transsphenoidal pituitary adenectomy. Four patients with hypothyroidism and TSH hypersecretion were treated with exogenous thyroid hormone. Post-therapy evaluation was carried out in 52 patients.

The majority of patients were evaluated in the Clinical Research Center. A few were evaluated as outpatients, or in cooperation with medicine and neurosurgical services.

Transsphenoidal pituitary adenectomy was performed by our neurosurgical staff in the manner previously described.[32] In three patients, it was performed twice. A few patients had undergone unsuccessful surgery and/or radiotherapy.

All patients had a thorough history and physical examination before and after surgery. Endocrine evaluation was performed as follows:

(1) Thyroid supplementation was discontinued at least six weeks prior to evaluation. Glucocorticoid replacement was discontinued for at least 48 hours and, in most cases, two weeks or more. Other medications including oral contraceptives were discontinued for at least two weeks.

(2) Baseline studies drawn fasting at 8 AM the first morning included thyroxine, TSH, cortisol, ACTH, prolactin, growth hormone, LH, FSH, and testosterone.

(3) The patients were given metyrapone, 2 to 3 gm orally, as previously described.[51] The following 8 AM plasma was obtained for cortisol, 11-deoxycortisol, and ACTH.

(4) Levodopa, 500 mg, was given orally at 8 AM and samples obtained serially thereafter for growth hormone and/or prolactin.

(5) Phenothiazine stimulation was accomplished by chlorpromazine hydrochloride, 50 mg parenterally, or perphenazine 8 mg orally, and serial measurement of serum prolactin thereafter.

(6) Thyrotropin releasing hormone (TRH) was given to one patient, 500 µg intravenously, and serial samples for TSH and prolactin obtained thereafter.

(7) Glucose tolerance test with 100 gm oral glucose was performed in the usual manner, with serial samples for growth hormone and glucose obtained for three hours thereafter.

(8) Clomiphene citrate was given orally, 50 mg, twice a day for one week, with LH and FSH determinations at the conclusion.

(9) In one patient with LH hypersecretion, ethinyl estradiol, 100 µg, was given by mouth each day for one week, and LH, FSH, and estradiol levels measured before and after.

(10) One and four mg dexamethasone suppression tests were completed as previously described.[8]

(11) Insulin hypoglycemia was induced and growth hormone measured as previously described.[52]

All hormones were measured by radioimmunoassay. Glucose was measured by the glucose oxidase reaction.

In most cases, visual field examinations were performed by the Ophthalmology Service. In a few patients, gross confrontation was felt to be adequate.

PROLACTIN-SECRETING ADENOMATA

Thirty-three patients with hyperprolactinemia and evidence of pituitary adenoma were evaluated. A summary of their initial clinical presentations is presented in Table 2.

Preoperative

All premenopausal females presented with one, or a combination of amenorrhea, galactorrhea, or infertility, except for two acromegalic patients who were seen initially for arthritic complaints. Post-menopausal females presented with headache or visual problems, as did one male. Complaints of the other two males centered around decreased gonadal function.

TABLE 2

PATIENTS WITH PROLACTIN – SECRETING ADENOMATA

Patient No, Age, Sex	CC	Occurrence	A	G	VF	X-ray	TSPA	Path
1, 28, F	A & G	spontaneous	+	+	abn	skull films	3/8/74	chrom
2, 27, F	A & infert	spontaneous	+	+	wnl	skull films	5/13/75	chrom + acid
3, 37, F	A & G	? spontaneous	+	+	wnl	sellar tomos	2/4/77	chrom
4, 28, F	A	post partum	+	+	wnl	skull films	8/31/76	--
5, 24, F	A & G	post BCP	+	+	wnl	skull films	8/31/76	chrom
6, 26, M	hypo-androgen	pre-puberty	–	–	wnl	pneumo wnl	6/4/76	chrom
7, 26, F	A & G	post partum	+	+	wnl	skull films	4/30/76	chrom
8, 27, F	A & G	post partum	+	+	wnl	pneumo	9/2/75	chrom
9, 23, F	A	spontaneous	+	+	wnl	skull films	6/3/75 5/25/76	chrom chrom
10, 32, F	A & G	post partum	+	+	wnl	sellar tomos pneumo - wnl	8/21/74	chrom
11, 37, F	swelling, face, joints	post partum?	–	+	wnl	--	6/28/76	acid
12, 21, F	A & G	post partum	+	+	wnl	skull films	12/1/76	--
13, 23, F	A	spontaneous	+	+	abn	skull films	9/21/76	chrom
14, 22, F	A	post partum	+	+	wnl	skull films	9/24/76	chrom

TABLE 2

PATIENTS WITH PROLACTIN – SECRETING ADENOMATA (Cont'd)

Patient No, Age, Sex	CC	Occurrence	A	G	VF	X-ray	TSPA	Path
15, 48, F	arthritis	spontaneous	–	PM	wnl	sellar tomos	1/25/77	chrom
16, 30, F	A & G	post BCP	+	+	wnl	skull and pneumo wnl, except partially ES	6/8/76	chrom
17, 54, F	headache	spontaneous	–	PM	wnl	skull films	8/23/74	chrom
18, 26, F	A & G	post BCP	+	+	wnl	sellar tomos	12/3/74	chrom
19, 26, F	A & G	spontaneous	+	+	abn	pneumo	1/21/75	baso
20, 33, F	A	spontaneous	+	+	wnl	sellar tomos	1/21/77	--
21, 21, F	A & G	spontaneous	+	+	wnl	skull films	5/7/74 11/5/76	chrom chrom + acid
22, 59, M	visual probs	spontaneous	–	–	abn	skull films	3/19/76	chrom
23, 31, F	A	spontaneous	–	+	wnl	skull films	1/20/76	chrom
24, 56, F	visual probs	spontaneous	–	PM	abn	skull films	5/21/76	chrom
25, 33, F	A	spontaneous	–	+	wnl	sellar tomos	5/24/77	chrom
26, 55, M	impotence gynecomas	spontaneous	–	–	abn	sellar tomos	--	--
27, 18, F	G	spontaneous	+	+	?abn	sellar tomos	--	--

TABLE 2

PATIENTS WITH PROLACTIN – SECRETING ADENOMATA (Cont'd)

Patient No, Age, Sex	CC	Occurrence	A	G	VF	X-ray	TSPA	Path
28, 22, F	A & G	post BCP	+	+	wnl	sellar tomos wnl w/o pneumos	--	--
29, 59, F	visual probs	spontaneous	–	PM	abn	skull films	--	--
30, 60, F	headache	spontaneous	–	PM	wnl	skull films	--	--
31, 21, F	A & G	spontaneous	+	+	wnl	skull films	--	--
32, 26, F	infertility	post partum	+	+	wnl	sellar tomos wnl w/o pneumo	--	--
33, 24, F	A & G	spontaneous	+	+	wnl	sellar tomos wnl w/o pneumo	--	--

Abbreviations: CC = chief complaint A= amenorrhea G = galactorrhea VF = visual fields TSPA = transsphenoidal pituitary adenectomy Path = pathological dx. (chrom = chromophobe adenoma, acid = acidophilic adenoma, baso = basophilic adenoma). BCP = birth control pills (estrogen-containing) PM = postmenopausal wnl = normal abn= abnormal

Note: Patients 2, 10, and 11 had coincident growth hormone hypersecretion. Patient 28 had coincident LH hypersecretion.

Of the 25 premenopausal females evaluated, 13 (52%) developed symptoms spontaneously, eight (32%) following pregnancy, and four (16%) after taking oral contraceptives. All had amenorrhea, and 22 (88%) had galactorrhea. None of the males or postmenopausal females had galactorrhea.

Visual field abnormalities were suspected or confirmed in eight patients prior to surgery. X-ray evaluation with plain x-ray of the skull suggested the diagnosis of pituitary adenoma in 17 (52%), and sella turcica tomography was required in eight patients (24%) one of whom had a normal pneumoencephalogram later. Pneumoencephalogram was necessary for diagnosis in three patients (9%). Six of 24 patients (25%) who underwent pneumoencephalography had evidence of partial or complete empty sella, and one patient had a calcified pituitary adenoma.

Preoperative endocrine evaluation is shown in Fig. 1 and Table 3. All patients except one (10) had persistently elevated prolactin levels. Although baseline levels in this patient were only high normal, there was inadequate suppression of prolactin by levodopa. In patient 2, the prolactin assay was performed in a laboratory, with a normal range of 4 to 28 ng/ml.

All patients who were non-acromegalic had undetectable GH. The three patients with acromegaly had persistently elevated GH, unsuppressible by glucose.

A levodopa test was performed on 15 patients with prolactin-secreting pituitary adenomata and eight other patients ("normal controls") with non-pituitary diseases. In six "normal controls," GH rose appropriately after levodopa. Two had undetectable levels of GH before and after the drug, patients with Arnold-Chiari malformation and idiopathic hirsutism respectively, but no other evidence of pituitary malfunction.

In nine patients with prolactin-secreting pituitary adenomata, three had a normal GH elevation after levodopa. Insulin hypoglycemia produced normal GH elevation in two other patients.

Prolactin was measured after levodopa in five "normal controls" and 13 patients with prolactin hypersecretion.

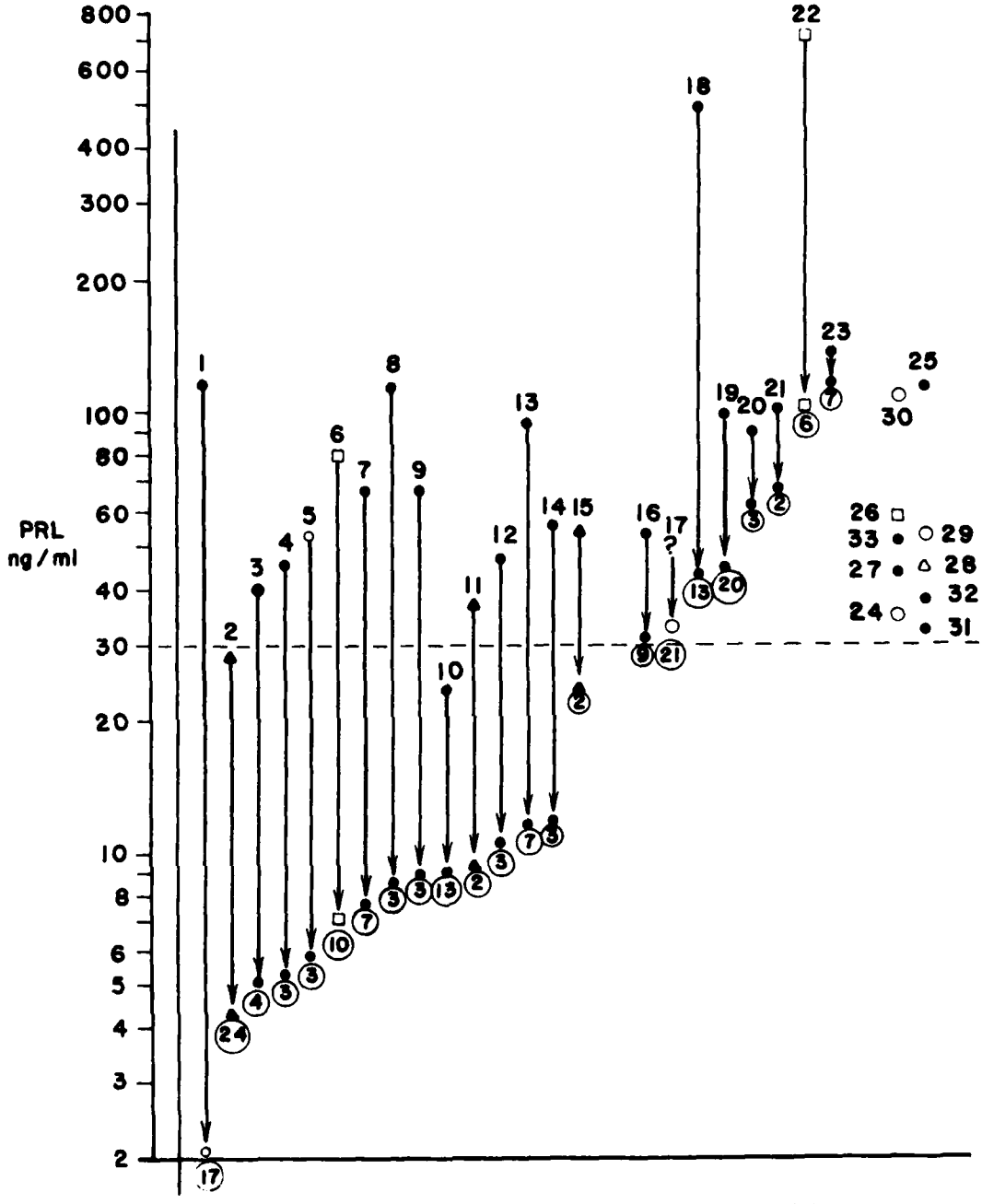

Figure 1. Baseline prolactin (PRL) levels before and after surgery. Normal range is 2-30 ng/ml. Arrow points to postoperative value. Numbers identify patient. Numbers in circles at end of arrow indicate months postoperative. ○ = Post-menopausal. □ = Male. ▲ = Acromegalic. △ = LH-secreting adenoma.

TABLE 3

PROLACTION-SECRETING ADENOMATA (PRE-OPERATIVE)

Patient No.	GH ng/ml	LH mIU/ml	FSH mIU/ml	T ng/dl	T_4 μg/dl	TSH μU/ml	S $\overline{p}$ met μg/dl	ACTH $\overline{p}$ met pg/ml
1	–	–	–	–	–	–	–	–
2	72	<1.5	<1.5	–	9.4	–	10.8	369
3	<2	1.9	8.4	–	–	–	12.1	–
4	<2	1.6	2.4	312	6.9	3.2	11.4	200
5	<2	13.6	4.9	210	6.9	4.4	11.6	313
6	<1.8	1.8	<1	226	11.2	1.2	15.1	604
7	–	26.6	10.1	–	9.6	2.3	13.8	–
8	<1	1.2	<1.8	117	7.2	4.3	17.2	454
9	<2	1.1	1.8	195	7.8	2.6	14.7	205
10	<2	–	–	–	6.3	–	9.7	–
11	50	7.7	5.4	144	6.7	5.6	11.7	529
12	<2	4.0	6.4	203	7.2	4.4	5.8	130
13	<2	7.5	5.6	206	8.4	10.9	8.9	317
14	<2	18.9	6.1	199	11	1.6	16.7	–
15	22	29	32	180	6.4	<1.3	14.1	170
16	<2	16.5	10.0	172	7.9	2.6	14.2	516
17	–	–	–	–	10.3	–	–	–
18	1.0	2.8	3.3	–	7.7	2.5	19.0	–

TABLE 3

PROLACTIN-SECRETING ADENOMATA (PRE-OPERATIVE) Cont'd

Patient No.	GH ng/ml	LH mIU/ml	FSH mIU/ml	T ng/dl	T_4 μg/dl	TSH μU/ml	S $\bar{p}$ met μg/dl	ACTH $\bar{p}$ met pg/ml
19	<1.8	29	7.0	236	–	–	20.9	–
20	–	–	–	–	–	3.7	–	–
21	–	21	–	–	5.7	–	17.1	–
22	<2	3.4	3.3	89	7.6	3.8	7.3	190
23	<2	2.2	<0.5	276	7.8	1.5	18.6	616
24	<2	6.2	6.7	114	4.8	3.1	3.5	–
25	<2	8.0	8.0	224	6.3	7.0	3.4	643
26	<2	4.0	6.9	363	5.8	7.8	14.1	868
27	<2	2.3	9.3	105	5.9	1.6	10.6	295
28	<2	23.4	9.1	137	7.1	5.9	10.2	362
29	<2	3.2	14.3	170	4.8	2.6	2.9	52
30	<2	<1	<1	122	5.8	2.9	2.6	32
31	<2	9.2	7.0	–	8.9	7.0	15.0	201
32	<2	3.2	2.3	251	8.1	7.5	15	620
33	9.6	5.8	8.3	147	7.1	7.0	–	414
Normal	<10	<1-30	<1-30	<150 F >300M	5-13.7	<10	7-20	>190

T = testosterone met = metyrapone

All five normal controls suppressed to less than 36% of baseline levels. All but two of prolactin hypersecretors failed to suppress to less than 38% of baseline, but four suppressed to between 38% and 50%. Of the two that did suppress normally, one suppressed from 30.4 to 7.0 ng/ml and the other from 52 to 16.5 ng/ml.

Phenothiazine stimulation was carried out on five "normal controls" and on 15 patients with prolactin-secreting pituitary adenomata. After phenothiazines, four of the "normal controls" had elevation of prolactin to greater than 200% of baseline. The one who failed to stimulate was the patient with Arnold-Chiari malformation. Only 1/12 patients (8%) with prolactin-hypersecretion stimulated more than 150% of baseline (from 27.2 to 68 ng/ml), the same patient who had suppressed to 7 ng/ml after levodopa.

Hypothyroidism was found in only 2/30 (6%) of patients. Four of 29 patients had decreased adrenal function. Testosterone was measured in 16 premenopausal females, 11 (69%) having elevated values. Elevated values were also found in two of our postmenopausal females. Values for two of three males were abnormally low.

Although normal values for gonadotropins are considered to be < 30 mIU/ml, we consider < 5mIU/ml to be low-normal. Ten of 22 premenopausal females (45%) fell into this low-normal category for LH, and 8/22 (36%) for FSH. In the three males tested, all had low-normal LH, and two of three patients had low-normal FSH as well. Of three post-menopausal females examined, two had low LH and one low FSH, considering their hypogondal states.

One patient with an unexpectedly high preoperative LH did not suppress significantly (from 33.6 to 23.7 mIU/ml) after one week of oral ethinyl estradiol, 100 µg daily. FSH suppressed from 12.4 to 2.8 mIU/ml and estradiol from 93 to 29 pg/ml during the test.

LH and FSH were measured after clomiphene citrate in 12 "normal controls." Nine (75%) stimulated to greater than 150% of baseline LH and seven (58%) of FSH. When LH and FSH were used together, nine (82%) had elevation of gonadotropin to greater than 150% of baseline after clomiphene.

Seventeen patients were given clomiphene citrate. Of 13 premenopausal females, nine (69%) had an LH rise to more than 150% of baseline and eight (62%) of FSH. In 10 (77%), either LH or FSH rose appropriately. One of the patients with a normal response had an LH-secreting pituitary adenoma (Patient 28). Her LH rose from 23.4 to 46.9 mIU/ml and FSH from 9.1 to 14 mIU/ml after administration of clomiphene. Of two males tested, one had a normal response to clomiphene. In two postmenopausal females with already high LH and FSH, these rose to greater than 150% of already elevated baseline values. The third had complete gonadotropin deficiency and no response to clomiphene.

Postoperative

Twenty-five of the 33 patients underwent transsphenoidal pituitary adenectomy. Two underwent the procedure for a second time, after an initial failure. Pathological diagnosis was chromophobe adenoma in 22 (88%), acidophilic adenoma in three (12%), and basophilic adenoma in one, in 24 available pathological reports. (Two of the above adenomata had both chromophobe and acidophilic elements.)

Postoperative complications in all the hormone-secreting adenomata operated, including ACTH and GH-secreting adenomata (to be discussed later), were minimal and included no mortality, meningitis, permanent diabetes insipidus (except in one patient in whom it occurred initially, secondary to previous transsphenoidal surgeries), hemorrhage, cranial nerve palsies, or palatal diastasis. There was one case of CSF rhinorrhea, and five cases of nasal septal perforation, several requiring repair. No nasal septal perforation occurred in the last 20 cases following use of new operative techniques.

In the patients with prolactin-secreting pituitary adenomata, cessation of amenorrhea and galactorrhea paralleled normalization of prolactin levels in all cases. Six patients became pregnant spontaneously shortly after surgery.[53] Four delivered healthy term infants, one is still pregnant, and one underwent abortion unrelated to pituitary disease.

In five patients with abnormal visual fields preoperatively, three had improvement and two complete normalization. No patients previously normal became abnormal. Two patients with normal x-ray studies (other than partial empty sella in one) underwent surgery, and an adenoma was found in each.

Postoperative endocrine evaluation was completed from two to 24 months after surgery (Fig 1 and Table 4).

In 16 of 23 (70%) patients evaluated, prolactin was in the normal range. In addition, one patient with minimally elevated value of 32.5 ng/ml was pregnant during the measurement.

Levodopa was given to four patients. None suppressed to less than 36% of baseline prolactin, although three suppressed to less than 50%. All had normal baseline prolactin, and two had previously suppressed to 57% and 58% of baseline prolactin values. The fourth, who suppressed to only 58% of baseline, had baseline value of 67 ng/ml and was an operative failure.

Six patients were given phenothiazines, with only one stimulating to more than 200% (from 27.2 to 68 ng/ml). Four of those who failed to stimulate had also failed preoperatively, only one having an elevated baseline prolactin, and another (Patient 6, an operative cure) also failing to stimulate after TRH.

Of non-acromegalic subjects, most had undectable GH after surgery, five had values in the measurable normal range, and one a slightly elevated value. Two acromegalic subjects had normal GH, with GH remaining elevated in the other.

No patients were clearly hypothyroid after surgery, although one had a borderline low T_4 value. Of the two previously low, one was not reevaluated after surgery, and the other has refused treatment.

Adrenal function became abnormal in four patients previously normal; however, one of these patients had been on supraphysiologic prednisone therapy. Only one previously abnormal patient underwent surgery, and was normal postoperatively.

TABLE 4
PROLACTIN-SECRETING ADENOMATA (POST-OPERATIVE)

Patient No.	GH ng/ml	LH mIU/ml	FSH mIU/ml	T ng/dl	T_4 μg/dl	TSH μ U/ml	S $\bar{p}$ met μg/dl	ACTH $\bar{p}$ met pg/dl
1	7.8	7.8	–	–	9.6	–	9.8	158
2	8.0	>2620	<1.0	–	12.5	5.1	15.0	–
3	–	16.0	7.2	–	5.7	7.1	7.5	–
4	<2	7.8	8.4	–	–	7.1	14.9	–
5	3.1	27	–	–	5.9	4.6	13.5	–
6	<2	5.2	12.4	672	8.5	2.3	10.9	368
7	<2	5.5	7.4	15	6.8	2.9	0	193
8	4	26	4.4	–	7.2	1.1	11.9	269
9	12	17.3	51.4	–	13.3	12	1.9	173
10	<2	9	10	–	6.9	–	18.9	–
11	6.7	5.8	8.3	–	8.7	7.0	11.0	692
12	<2	20.4	10.5	–	8.0	4.4	13.5	845
13	<2	10.5	3.4	–	12.8	3.5	12.3	558
14	8.3	7300	<1	87	8.2	9.8	–	>600
15	13	15	15	–	8.7	4.3	10.0	338
16	<2	–	–	–	–	3.0	–	–
17	<2	–	–	–	9.5	–	9.5	175
18	<2	>2500	3.6	–	7.0	3.6	11.8	190
19	15	12	4	–	7.9	5.6	12.3	327
20	<5	3.2	6.2	–	5.7	4.8	10.0	272

TABLE 4

PROLACTIN-SECRETING ADENOMATA (POST-OPERATIVE) Cont'd

Patient No.	GH ng/ml	LH mIU/ml	FSH mIU/ml	T ng/dl	T_4 μg/dl	TSH μU/ml	S $\bar{p}$ met μg/dl	ACTH $\bar{p}$ met pg/dl
21	<2	8	8.5	53	5.8	6.1	3.1	253
22	<2	3.3	1.5	128	5.1	5.5	4.0	170
23	<2	4.0	5.3	–	8.8	3.3	15.9	396
24	–	–	–	–	–	–	–	–
Normal	<10	<1-30	<1-30	<150F >300M	5-13.7	<10	7-20	>190

T = testosterone met = metyrapone

Of eight premenopausal females with elevated testosterone preoperatively, only two (operative cures) had normalization of testosterone. Four other patients who were cured maintained high testosterone levels (one of these patients was pregnant) as did two operative failures.

Of two males who had low testosterone preoperatively, the level returned to normal after successful surgery in one and remained low in the other (an operative failure).

All but one premenopausal female had LH value greater than 5 mIU/ml postoperatively. Three patients (one with normal and two with low-normal preoperative LH levels) had extremely high postoperative LH. As all three were pregnant at time of evaluation, the values reflect cross-reaction with chorionic gonadotropin.

Of the three males, one had normal LH postoperatively. The other two underwent surgery, and had continued low-normal values.

In patients with prolactin-secreting adenomata, FSH tended to parallel LH values. An exception were the three patients who became pregnant, in whom FSH dropped still lower, coinciding with LH rise, and consistent with normal pregnancy.[54]

Thirteen patients were given clomiphene. Only 2/10 premenopausal females stimulated to more than 150% of baseline LH and 0/8 to more than 150% of baseline FSH. One male stimulated only LH normally, and the other had no response to clomiphene.

ACROMEGALY

Twenty patients with growth hormone-secreting pituitary adenomata were initially evaluated (Table 5.)

Preoperative

The male-to-female ratio was 2:3 with arthropathy the most common presenting complaint. Other common complaints

TABLE 5

PATIENTS WITH GH-SECRETING ADENOMATA

Patient No., Age, Sex	CC	VF	HT	DM	X-rays	Other	TSPA	Path
34,54,M	arthritis	N	+	–	sellar tomos	carpal tunnel; Hx TFH and RRX (proton beam)	5/17/74	chrom
2,27,F	amenorrhea; infertility	N	–	?	skulls	hirsutism	5/13/75	chrom + acid
35,30,F	carpal tunnel	N	–	–	skulls	arthritis, amenorrhea; post-op RRX	3/25/75	chrom + acid
36,30,F	oligomenorrhea	N	–	–	skulls	headache; TSPA x 2 RRX	10/30/73	chrom
37,49,F	sweating	N	–	+	skulls	carpal tunnel	12/4/73	chrom
38,52,M	arthritis	N	–	–	skulls	emot labil; Hx RRX	10/9/73	chrom
39,60,M	headache; macroglossia	?	+	–	skulls	carpal tunnel; arthritis; dec libido Hx RRX	2/8/74	acid
40,36,F	carpal tunnel; headache	N	–	?	skulls	hair loss; A & G arthritis, sweating, emot lab	3/4/77	chrom

TABLE 5

PATIENTS WITH GH-SECRETING ADENOMATA (Cont'd)

Patient No., Age, Sex	CC	VF	HT	DM	X-rays	Other	TSPA	Path
41,64,F	arthritis	?N	+	+	skull	hyperpigmentation; hoarseness, glaucoma RRX 1963	9/22/72	chrom
11,46,M	acral enlargement visual problems	N	–	–	skulls	carpal tunnel; suicide	11/20/72	chrom
42,27,F	swelling face, joints	N	–	–	pneumo	A & G	6/28/76	acid
43,58,M	diff voiding	N	–	–	skull wnl pneumo ES	arthritis	10/7/75	nothing
15,49,F	arthritis	N	+	–	sellar tomos pneumo ES	carpal tunnel; sweating	1/25/77	chrom
44,52,M	acral enlargement	N	–	+	pneumo	carpal tunnel	10/3/72	chrom
45,34,F	HA, nausea	A	–	–	skulls	arthritis; G; emot labil TSPA 1973;post-op RRX	6/26/73 11/12/74	acid acid
46,33,F	headache	A	–	+	skulls	amenorrhea, carpal tunnel; fatique	3/4/75	chrom

TABLE 5

PATIENTS WITH GH-SECRETING ADENOMATA (Cont'd)

Patient No., Age, Sex	CC	VF	HT	DM	X-rays	Other	TSPA	Path
47,55,F	acral enlargement	N	–	–	skulls	carpal tunnel; arthritis	6/9/73	chrom
48,42,F	carpal tunnel	A	–	–	skulls	A & G; arthritis; headache	4/2/74	chrom + acid
49,46,M	arthritis	N	?	–	skulls	numbness, tingling	5/10/77	chrom + acid
50,42,M	diplopia, headache	N	–	–	skulls	dizziness	––	––

Abbreviations: CC = chief complaint VF = visual fields HT = hypertension N = Normal TFH = Transfrontal hypophysectomy
RRx = radiotherapy TSPA = transsphenoidal pituitary adenectomy A & G = amenorrhea and galactorrhea emot lab = emotional lability

included paresthesias associated with carpal tunnel syndrome, and amenorrhea in premenopausal females. All patients had some signs of acral enlargement and soft tissue increase.

Visual fields were definitely abnormal preoperatively in three of 19 patients (15%) and questionable in one. Hypertension was found in four (20%) and suspected in another. Diabetes was noted in three (15%), and borderline in another.

Simple x-rays of the skull permitted us to make a diagnosis of pituitary disease in 15 (75%) of patients with sellar tomograms necessary in two (10%), and pneumoencephalogram in two (10%). One patient had normal skull x-rays and empty sella on pneumoencephalogram, and two other patients showed partially empty sellas.

Preoperative endocrine evaluation is shown in Fig 2 and Table 6. Baseline GH was considered elevated in all patients. After glucose in 14 patients none suppressed to more than 50% of baseline, and none into the normal range, except for one patient (13) who suppressed from 7 to 5 ng/ml (with normal range of 0-8 ng/ml for this sample).

Baseline prolactin was measured in 11 patients. It was elevated in three patients (27%). In four other patients who had growth hormone elevation after surgery, prolactin was not elevated. If these two groups are combined, only 3/15 (20%) of patients can be considered to have coincidental prolactin secretion from adenomata. Two patients who complained of amenorrhea and galactorrhea had normal prolactin levels.

Of 15 patients assessed, only one (6%) was hypothyroid. Two of 10 patients (20%) had evidence of secondary adrenal insufficiency. Gonadotropins were measured in 11 patients. The three postmenopausal females had appropriately elevated LH and FSH values, while in four of five premenopausal females, LH and FSH values were low-normal, i.e., less than 5 mIU/ml. The three males appeared normal.

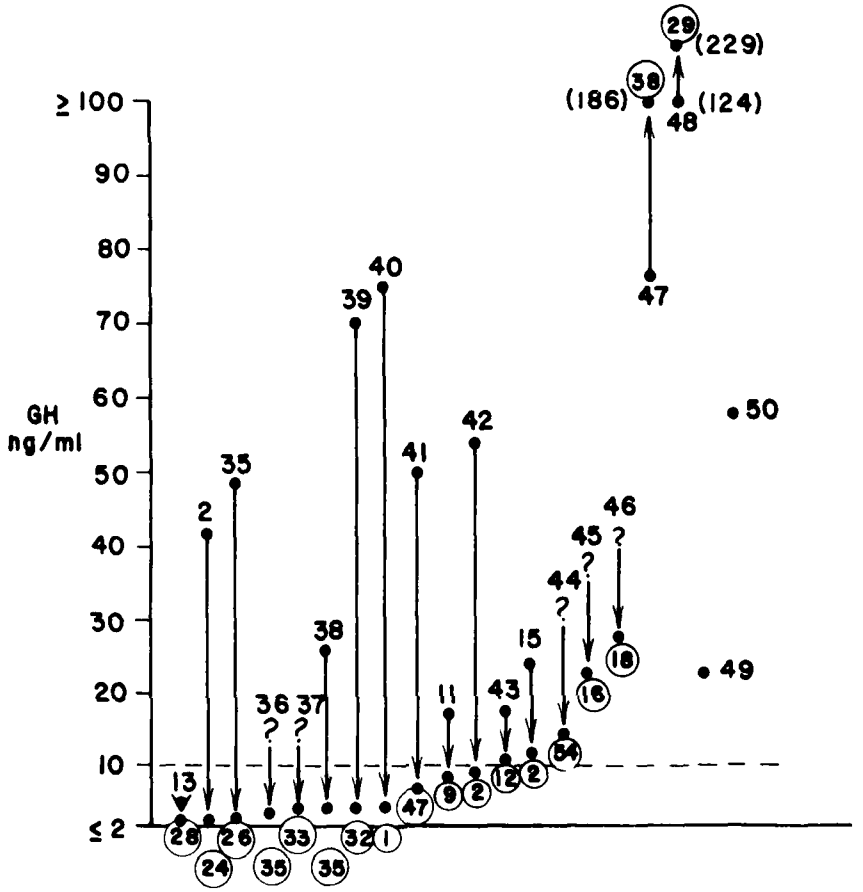

Figure 2. Growth hormone (GH) levels after suppression with oral glucose, shown before and after surgery. Normal range for baseline growth hormone is less than 10 ng/ml. Arrow points to post-operative value. Numbers identify patients. Numbers in circles at end of arrows indicate months postoperative. Numbers in parentheses = GH value if greater than 100 ng/ml.

TABLE 6

ACROMEGALY (PRE-OPERATIVE)

Patient No.	PRL ng/ml	LH mIU/ml	FSH mIU/ml	T_4 μg/dl	TSH μU/ml	S $\bar{p}$ met μg/dl	ACTH $\bar{p}$ met pg/dl	GH ng/ml
34	–	–	–	5.2	–	–	–	8
2	28*	<1.5	<1.5	9.4	4.5	10.8	369	72
35	24.3	1.8	4.0	9.4	<1.6	9.4	–	114
36	–	–	–	–	–	–	–	26
37	–	–	–	–	–	–	–	60
38	–	–	–	8.7	–	–	–	26
39	–	–	–	–	–	–	–	64
40	17.1	3.6	2.6	8.8	4.3	7.3	500	82
41	12.0	24	26	14.4	4.8	10.7	–	64
11	5.4	5.8	3.6	8.1	–	–	–	34
42	37.3	7.7	5.4	6.7	5.6	11.7	529	50
43	–	–	–	2.4	4.3	0	55	12
15	54	29	32	6.4	<1.3	14.1	170	22
44	–	–	–	7.2	–	7.3	–	27

TABLE 6

ACROMEGALY (PRE-OPERATIVE (Cont'd)

Patient No.	PRL ng/ml	LH mIU/ml	FSH mIU/ml	T_4 μg/dl	TSH μU/ml	S $\bar{p}$ met μg/dl	ACTH $\bar{p}$ met pg/dl	GH ng/ml
45	–	–	–	–	–	–	–	65
46	22.4	3.3	10	9.2	3.9	–	–	53
47	5.2	29	33	16.2	<1.6	24.0	–	137
48	–	–	–	–	–	–	–	53
49	9.0	8.3	12.8	–	3.2	–	–	12
50	16.4	7.0	12.2	6.3	6.9	1.8	–	28
Normal	2-30	<1-30	<1-30	5-13.7	<10	>7	>190	>10

met = metyrapone

Postoperative

Nineteen patients underwent surgery; one was operated twice. Pathological diagnosis was chromophobe adenoma in 11 (58%), acidophilic adenoma in three (16%), and mixed chromophobe-acidophilic in four (21%). A pathological diagnosis was not obtained from one patient.

Postoperative complications have been discussed in the previous section. Abnormal visual fields in three patients were completely normal after surgery. One patient whose visual fields previously were normal had a questionable examination postoperatively. A repeat followup has not yet been obtained.

Hypertension and diabetes, when initially present, did not clear postoperatively.

Postoperative endocrine evaluation (Fig 2 and Table 7) was completed one to 54 months after surgery. Although 15 (83%) of patients had marked decrease of GH, three were in the abnormal range, resulting in a total chemical cure of only 67%. Three patients had an increase in GH postoperatively. After receiving glucose, only 1/11 patients with baseline GH in the normal range suppressed to less than 50% of baseline. Six suppressed somewhat, two had a paradoxical rise, and two had undetectable levels before and after glucose. No patients with elevated baseline GH (treatment failures) suppressed to less than 76% of baseline values, and two showed paradoxical elevations.

Of three patients with preoperative prolactin elevation, two had normalization of both GH and prolactin; GH remained elevated in one patient, but prolactin was in the normal range.

Postoperatively, the patient who initially was hypothyroid remained so. In addition, one patient who had low normal function became hypothyroid. He had previously undergone transfrontal surgery and heavy particle therapy. In summary, only 2/18 (11%) patients were hypothyroid after surgery.

The metyrapone test was abnormal in 6/18 (33%); however, only one of these six patients was evaluated preoperatively, at which time he was also found to be adrenally insufficient.

TABLE 7

ACROMEGALY (POST-OPERATIVE)

Patient No.	PRL ng/ml	LH mIU/ml	FSH mIU/ml	T_4 μg/dl	TSH μU/ml	S $\bar{p}$ met μg/dl	ACTH $\bar{p}$ met pg/dl	GH ng/ml
34	6.5	3	<1	2.2	3.4	0.5	52	<2
2	8.0	>2620	<1	12.5	5.1	15	–	<2
35	22.3	5.2	7.5	6.9	5.0	9.3	–	<2
36	18.1	6.2	8.9	6.5	3.5	3.6	195	7.8
37	88.8	2.4	6.5	7.9	8.5	9.9	358	<2
38	10.0	8.3	8.4	6.8	1.3	4.3	407	9.5
39	19.9	4.4	5.4	5.6	6.7	3.0	123	5
40	6.9	8.8	6.6	8.0	2.9	8.5	–	8.3
41	12.3	1.4	2.0	9.0	3.9	6.9	580	8.1
11	–	–	–	12.6	–	17.5	–	9.4
42	9.8	5.8	8.3	8.7	7.0	11.0	692	6.7
43	14.9	<1	<1	2.7	3.8	0.2	87	29
15	24.1	15	15	8.7	4.3	10.0	338	12.5
44	7.2	3.7	6.7	9.7	2.9	8.4	–	19

TABLE 7

ACROMEGALY (POST-OPERATIVE) (Cont'd)

Patient No.	PRL ng/ml	LH mIU/ml	FSH mIU/ml	T_4 μg/dl	TSH μU/ml	S p̄ met μg/dl	ACTH p̄ met pg/dl	GH ng/ml
45	18.8	4.6	5.3	8.6	4.6	4.4	–	10
46	7.9	7.6	5.7	9.0	–	12.4	406	39
47	5.5	25	35	13.7	–	8.9	250	163
48	19.1	36	12	11.6	4.2	22	771	120
Normal	2-30 *6-24	<1-30	<1-30	5-13.7	<10	>7	>190	<10

met = metyrapone

One patient had an inadequate metyrapone block. None of the eight patients with intact adrenal function documented preoperatively had deficiency postoperatively.

CUSHING'S DISEASE

Nine patients with Cushing's disease were evaluated and underwent transsphenoidal pituitary adenectomy. A summary of their initial clinical presentations is shown in Table 8.

Preoperative

All patients were female, ranging in age from 11 to 56. Visual fields were normal in the eight patients assessed. X-ray confirmation of pituitary disease was demonstrated in only four patients: two by skull x-ray, one by sella turcica tomography, and one by pneumoencephalography. One patient had the empty sella syndrome. In four patients, pneumoencephalography failed to demonstrate a pituitary adenoma, and in a fifth patient, sellar tomography was normal, with no pneumoencephalogram performed.

Diabetes mellitus was present in 3/9 (33%) patients, and hypertension in four (45%). Eight (89%) complained of hirsutism, and all complained of weight gain ranging from 13.5 kg to 27 kg. A Cushingoid habitus was found in all, although it was quite mild in some. Two patients became spontaneously pregnant prior to surgery, both delivering normally (although one infant had mild adrenal insufficiency).

Preoperative endocrine evaluation is shown in Table 9. None of the patients suppressed plasma cortisol to less than 12.1 µg/dl (normal less than 5 µg/dl) after 1 mg dexamethasone. High dose suppression achieved suppression to 50% or less of control cortisol in six of eight patients tested.

8 AM cortisol ranged from 8.3 to 28 µg/dl in the nine patients, with lack of diurnal variation in all. 11-deoxycortisol after metyrapone ranged from 6.4 to 25.5 µg/dl.

TABLE 8

Patient No, Age, Sex	Inital complaint	X-ray	VF	DM	HT	HI	WT	CU	other	TSPA	Path.
51,33,F	oligomennorhea	skulls	N	+	+	+	30	+		7/13/72	baso
52,33,F	wt. gain	pneumo--neg	N	–	–	+	60	+	(1)	8/3/73	normal
53,56,F	fatique, wt. gain	skulls pneumo--ES		+	+	–	+	+		6/28/74	baso
54,11,F	growth retard	pneumo--neg	N	?	–	+	+	+	(2)	8/30/74	normal
55,26,F	wt. gain	sellar tomos-N. no pneumo done	N	–	+	+	55	+		11/6/74	baso + chrom
56,19,F	oligomenorrhea	pneumo	N	+*	–	+	+	+	(3)	10/6/75	baso
57,38,F	diabetes	pneumo--neg	N	+	–	+	+	+	(4)	5/21/76	baso
58,28,F	headache	pneumo--neg		–	+	+	+	+		12/7/76	chrom
59,29,F	amenorrhea	sellar tomos	N	–	–	+	+	+	(5)	2/1/77	acid + chrom

X-ray = necessary to make diagnosis VF= visual fields DM = diabetes mellitus HT = hypertension HI = hirsutism WT = weight gain (+ or number indicates amount from baseline) CU = Cushingoid habitus TSPA = transphenoidal pituitary adenectomy path = pathological diagnosis

(1) pregnancy pre-op (2) kidney stones, treatment failure; underwent adrenalectomy (3) pregnancy post-op (4) pregnancy pre-op (5) headaches

TABLE 9

CUSHING'S DISEASE

PREOPERATIVE						POST-OPERATIVE		
Patient No.	F – AM	F – PM	F $\bar{p}$ 1 mg dex	F $\bar{p}$ 4 mg dex	S $\bar{p}$ met	F $\bar{p}$ 1 mg dex	S $\bar{p}$ met	T_4
51	8.3	8.5	16.6	5.4*	18.6	2.1	20.8	11.4
52	20.9	18.8	15.6	9.7	21.8	0.3	–	2.0
53	24.4	17.0	22.8	4.2	9.3	0.1	2.2	8.9
54	20.6	20.7	18.5	15.9	17.1	19.5	12.9	8.6
55	13.3	8.4	16.2	17.6	8.5	0.5	6.4	–
56	28	29	27.9	2.1	6.4	0	4.2	4.7
57	10.5	13.4	12.5	–	23.0	4.8	2.1	7.8
58	17.5	21.6	12.1	5.3	25.5	22.2	54.4	–
59	22.1	16.8	17.0	3.7	17.5	4.5	–	–
Normal	6-22	< 7	< 5	< 5	> 7	< 10	> 7	5-13.7

F = plasma cortisol μ g/dl S= plasma 11 - deoxycortisol μg/dl dex = dexamethasone
met = metyrapone T_4 = thyroxine μg/dl
* High Liddle test (2mg by mouth every six hours for 2 days)

Testosterone was elevated in only one of seven patients evaluated. Prolactin was measured in two patients and was normal. Two patients with normal LH and FSH complained of amenorrhea.

Postoperative

Complications of surgery have been discussed in a previous section. Visual fields remained normal in the eight patients assessed preoperatively. In the ninth patient, questionably abnormal fields were found after surgery, although only gross confrontation (normal) had been available preoperatively for comparison.

All patients who had diabetes mellitus showed improvement after successful surgery, and one patient who was normal preoperatively developed diabetes during a spontaneous pregnancy postoperatively. Hypertension corrected in three-fourths of patients, with a surgical cure of their adenoma.

Pathological diagnosis showed basophilic adenoma alone in 4/9 patients, basophilic adenoma combined with chromophobe elements or acidophilic elements in two others, and pure chromophobe adenoma in the seventh. Normal pituitary was submitted in the last two patients.

Postoperative endocrine evaluation is also shown in Table 9. A 1 mg dexamethasone suppression test was normal in eight (89%) patients, all showing improvement of their symptoms. One patient subsequently suffered a relapse and underwent bilateral total adrenalectomy for control of the disease. One patient was a surgical failure. 11-deoxycortisol was normal after metyrapone in only three of seven patients examined. In another patient, hydrocortisone replacement could not be discontinued because of her symptoms of adrenal insufficiency. In some patients, a long delay in recovery of adrenal function was noted in the postoperative months.

Only six patients had thyroid evaluation after surgery. Results were normal in four patients and abnormal in two, one of whom also had adrenal insufficiency.

TSH HYPERSECRETION

Four patients with primary hypothyroidism, TSH hypersecretion and x-ray evidence of pituitary enlargement were examined (Table 10). The two females presented with galactorrhea. One male presented with weakness thought to be secondary to adrenal insufficiency, and one with anosmia following head trauma. The third patient (62) presented with amenorrhea and galactorrhea following discontinuation of oral contraceptives. None of the three patients tested had visual field abnormalities. A skull x-ray was demonstrative of sellar enlargement in all patients. One had a partially empty sella.

TABLE 10

TSH HYPERSECRETION IN PRIMARY HYPO-THYROIDISM

Patient No, Age, Sex	Chief complaint	H	G	Other	VF	X-rays
60,39,M	fatigue, weakness	+	-	diabetes mellitus	N	skull films
61,21,F	galactorrhea	-	+	normal menses; G post oral contraceptives	-	skull films
62,31,F	A & G	-	+	--	N	skull films
63,39,M	anosmia, headache	-	-	dizziness	N	skull films partially ES

H = clinically hypothyroid, G = galactorrhea, VF = visual fields, A = amenorrhea, ES = empty sella

A summary of the endocrinological evaluation pre-treatment in all and post-treatment in three of the patients is shown in Table 11.

All patients initially had low T_4 and elevated TSH. The two patients with galactorrhea had elevated prolactin. Growth hormone was elevated in none. Gonadotropins were normal in all patients prior to therapy. Although three patients had normal adrenal function, one male (60) had pituitary adrenal insufficiency before therapy. He also had low testosterone. The other male had normal testosterone, and one female had slightly elevated testosterone.

All four patients were placed on adequate doses of replacement L-thyroxine. Post-therapy, the three patients who were re-evaluated showed TSH values in the normal range. Two patients had prolactin hypersecretion, which decreased to normal in one patient and decreased substantially in the other patient but was still elevated post-therapy. Gonadotropins remained unchanged, although testosterone improved in the patient whose value was low prior to therapy. This patient had little improvement in adrenal function, however.

TABLE 11
PRIMARY HYPO-THYROIDISM WITH PITUITARY ENLARGEMENT

Patient No.	T_4 μg/dl	TSH μU/ml	PRL ng/ml	GH ng/ml	LH mIU/ml	FSH mIU/ml	$S_{\bar{p}}$ met μg/dl	ACTH $_{\bar{p}}$ met pg/ml	T ng/dl
Pre-therapy									
60M	0.4	94	20.8	2.0	13.3	5.7	5.0	76	239
61F	3.2	75	34.4	<2	23.1	6.6	14.0	1238	152
62F	4.8	15	742	2	2.5	2.4	10.9	957	–
63M	2.9	77	6.1	<2	9.6	7.8	17.5	378	727
Post-therapy									
60	7.7	1.6	<2	4.5	13.8	9.1	3.9	113	956
61	11.2	3.2	21.3	–	21.0	8.9	–	–	–
62	16.3	1.7	84.5	3.0	2.5	4.9	12.2	–	–
Normal	5-13.7	<10	2-30	<10	0-30	0-30	7-20	>190	<150F >300M

met = metyrapone

DISCUSSION

Although pituitary adenomata have traditionally been classified according to their pathological staining qualities[55] (chromophobe, acidophilic or basophilic), with the advent of radioimmunoassay techniques to measure pituitary peptide hormones, a classification based on hormone secretion is more appropriate (Table 1).

In our series of 63 patients, 59 had autonomous hypersecreting pituitary adenomata. The other four had hypothyroidism and TSH hypersecretion. An additional 19 "nonfunctional" pituitary adenomata that were operated at the University of Utah Medical Center during the same period are not described in detail in this paper.

One cannot be certain, however, that the statistics adequately reflect the frequency of pituitary adenomata. Indeed, in routine autopsy series, small non-symptomatic adenomata are found in 20% of the population.[56]

In addition, many early growth hormone-secreting adenomata may not be diagnosed until the symptoms of hormone excess progress. Gonadotropin-secreting adenomata may be far more common than we realize, since the symptoms they create are difficult to diagnose. The prolactin-secreting adenomata appear to be the easiest to diagnose early, especially in premenopausal women, since symptoms of galactorrhea and amenorrhea are concrete complaints. The increased prevalence of prolactin-secreting pituitary adenomata may thus reflect increased rate of diagnosis now that a prolactin radioimmunoassay is available (Table 12).

The patients presenting late in their course were postmenopausal females and a male who presented only after the size of their adenomata caused symptoms. Two additional males presented with symptoms related to hypogonadism. One had been hypoandrogen for many years; the other had an identical twin brother, who looked more masculine than he.[57] Of the three patients with coincident

GH hypersection, two presented with complaints characteristic of acromegaly and one as a premenopausal female with typical symptoms of prolactin hypersecretion.

TABLE 12

PRESENTING COMPLAINT -- PROLACTIN HYPERSECRETION (n = 33)

prememopausal females	
amenorrhea	21*
galactorrhea	14
infertility	1
joint aches	1*
post-menopausal females	
joint aches	1*
headache	2
visual problems	2
males	
delayed puberty	1
gynecomastia	1
visual problems	1

* complaints of 3 patients with concurrent acromegaly

The 67% of premenopausal female patients who developed symptoms after pregnancy or after using oral contraceptives (Table 13) is in accord with published data of the increased frequency of these lesions in hyperestrogenemic states.[12] All of our patients had amenorrhea although amenorrhea may not always be present in these patients, and spontaneous pregnancies in women with hyperprolactinemia from a pituitary adenoma may occur. Of our patients, 84% had galactorrhea some time during their illness, although galactorrhea is not always initially present in such patients (Table 12). Our high percentage probably reflects the careful history and breast examination technique used.

Our patients with acromegaly presented similarly to those in earlier series.[1] Male-to-female ratio was lower in our group, perhaps because of early diagnosis in some premenopausal females complaining of amenorrhea. On physical examination, all patients had signs of soft tissue

thickening and acral enlargement, although in some patients these findings were very subtle. Neither hypertension nor diabetes mellitus was a frequent finding in our patients. Hypertension did not remit following successful surgery, and diabetes did not remit in two patients who were surgically cured. One may question the role that acromegaly had in causing the hypertension and diabetes, since both are common entities in the general population.

TABLE 13

ESTROGEN ASSOCIATION

(Prolactin-secreting, premenopausal F, *n*=25)

post-pregnancy	13	(52%)
post oral contraceptives	4	(16%)
spontaneous	8	(32%)

Examination of all patients with Cushing's disease showed the typical Cushingoid habitus. Most patients had hirsutism as well. Few complained of obesity per se. Three patients had diabetes mellitus; all were improved after successful surgery. Hypertension, a common finding, was corrected when surgery was successful.

Although pregnancy is not common in untreated Cushing's disease, two of our patients conceived while having overt symptoms. One had a normal delivery. The infant of the other patient showed some signs of adrenal insufficiency and had to be temporarily treated with glucocorticoids neonatally. A third patient became pregnant spontaneously postoperatively.

Visual fields preoperatively were most often normal. They were confirmed or suspected to be abnormal in 24% of patients with prolactin-secreting adenomata, 15% of patients with acromegaly, and in none with Cushing's disease. After successful surgery, all improved or became normal. A

patient with unsuccessful surgery and another who refused surgery experienced deterioration of their vision.

Microadenomata were common, especially in patients with prolactin or ACTH hypersecretion. Routine skull x-rays were abnormal in only 22% of ACTH-secreting adenomata, 51% of prolactin-secreting adenomata, and 75% of growth hormone-secreting adenomata. These results undoubtedly reflect the amount of time required for these types of patients to present medically. (Patients with acromegaly have the disease longest before seeking medical advice.) In seven patients, even pneumoencephalography failed to demonstrate an adenoma; all patients went to surgery on the basis of endocrinologic criteria. An adenoma was found in all patients except in two with Cushing's disease. The adenoma may have been missed at surgery or ectopically located, as occasionally occurs.[58] Partial or complete empty sella syndrome was a finding in 24% of prolactin-secreting adenomata, 10% of growth hormone-secreting adenomata, and in one ACTH and TSH hypersector each. This finding agrees with the incidence in other series and may well be unrelated to the pituitary disease, since a similar frequency of empty sellas may be found in the normal population.[59] One patient (30) had a calcified pituitary adenoma. Calcification has been found in as many as 6.6% of pituitary adenomata examined by sensitive techniques.[60]

Surgical cure was assessed in all patients by normalization of hormone levels postoperatively. In the case of ACTH-secreting adenomata, assessment was made following dexamethasone suppression, since baseline ACTH and cortisol values in these patients often overlap with normal subjects. The rates of cure are summarized in Table 14.

TABLE 14

POST-OPERATIVE HORMONE NORMALIZATION IN PITUITARY ADENOMATA

HORMONE HYPERSECRETED	NUMBER PATIENTS	NUMBER NORMALIZED	% NORMALIZED
PRL	23	16	70
GH	18	12	67
ACTH	9	8	89

It must be stressed, however, that in some cases total chemical remission was not attempted. In five patients with prolactin-secreting adenomata and nine with GH-secreting adenomata, there was no hope for complete adenoma excision, and only decompression and lowering of hormone levels was attempted. Clearly lowering hormone levels, even if not into the normal range, had beneficial effects on many of these patients. It has previously been shown in acromegaly that radiotherapy[47] or medical therapy[40] often does not reduce growth hormone levels to normal but may result in symptomatic improvement.

Preoperatively, only 8% of patients were hypothyroid. One of these patients had Cushing's disease and the low T_4 may have been secondary to increased glucocorticoids. Postoperatively, a similar percentage of hypothyroidism was found (9%), including four patients who were known to be euthyroid preoperatively. The procedure itself may have caused pituitary TSH deficiency in these patients; however, some had received prior radiotherapy and/or surgery which may have played a role.

Adrenal insufficiency was not common in any group of patients preoperatively except in a few patients with very large adenomata. Postoperatively in prolactin and GH-secreting adenomata, 34% had some adrenal insufficiency. However, only four patients previously documented to be normal preoperatively became abnormal. Again, the remaining patients may have had adrenal insufficiency prior to surgery caused by pituitary compromise from the large size of their adenomata or from previous surgery or radiotherapy. Adrenal insufficiency was found by preoperative metyrapone testing in approximately 75% of patients with Cushing's disease. However, some of these patients are known to take many months for pituitary-adrenal axis recovery, and not all of our patients have yet had an adequate recovery period. (See Tyrrell, pp. 5-18, this publication.)

Baseline gonadotropins were low in most premenopausal patients with prolactin-secreting pituitary adenomata, and some with growth hormone-secreting lesions. In many patients with larger adenomata, frank gonadotropin deficiency was certainly present, for there was no return of function after surgery. However, in the majority of

patients with prolactin hypersecretion, successful removal of the adenoma resulted in normalization of gonadotropin levels.

Stimulation and suppression tests were used in many patients. In patients with Cushing's disease, the dexamethasone suppression test was a mainstay of diagnosis.

In growth hormone-secreting adenomata, suppression with glucose was useful. Several patients with borderline growth hormone values baseline did not suppress after a glucose load in contrast to most normal subjects who suppress to less than 1 ng/ml after glucose.[61]

Levodopa suppression of prolactin was assessed in patients with and without prolactin-secreting adenomata. Although most normal subjects suppressed well with this agent, so did some of the patients with adenomata, making the test of limited use in diagnosing small adenomata, in agreement with recent literature.[12] Phenothiazine stimulation appeared a more effective means of identifying adenomata. However, some normal subjects did not stimulate and some patients with adenomata did, thereby limiting the usefulness of this test for diagnosing prolactin-secreting pituitary adenomata. Our current approach to diagnosis is to measure three baseline prolactin levels and use other x-ray and historical data.

Postoperatively, none of the patients assessed suppressed well to levodopa, and only one stimulated after phenothiazines. One who failed to stimulate after phenothiazines also failed to do so after TRH. These data suggest that even after surgical cure, i.e., normalization of prolactin levels, some hypothalamic-pituitary defect may still be present in these patients. Similarly, few patients with cured growth hormone-secreting pituitary adenomata postoperatively suppressed with glucose as well as was expected of normal subjects. In contrast, most patients cured of Cushing's disease suppressed well after dexamethasone.

Clomiphene stimulation was not useful in diagnosing prolactin-secreting pituitary adenomata. In general, only those patients with mid-normal range LH and FSH stimulated

adequately. Those with lower normal values, i.e., less than 5 mIU/ml, were difficult to stimulate. However, in the few who could elevate LH and FSH substantially, clomiphene could have therapeutic benefit in terms of inducing ovulation.

Several of our patients with hyperprolactinemia complained of hirsutism preoperatively. A similar complaint by patients, most of whom had normal testosterone values, has been reported.[62] Sixty-nine percent of pre-menopausal females with hyperprolactinemia in our series had elevated testosterone values, some of which did not return to normal even after a surgical cure. The initial elevations may have been caused by a hormone other than testosterone (possibly dehydroepiandrosterone (DHEA) sulfate), rather than testosterone which would correlate with recent reports of increased DHEA sulfate in these patients.[63,64] Why some nonpregnant patients who were otherwise cured continued to have elevated testosterone values awaits results of an investigation which is currently underway.

The low testosterone in the males was caused by depressed gonadotropins secondary to hyperprolactinemia.[15] In the one patient who was cured, testosterone returned to normal. In the patient who was not cured, testosterone did not return to normal.

Pathological diagnosis of adenomata removed at surgery was carried out by routine staining techniques. The fixed sections, although usually showing pituitary adenoma, did not identify the type of hormone secretion. It is apparent that all types of hormone-secreting adenomata may stain "chromophobe" because of a high turnover rate with diminished storage in cytoplasmic granules. The question of which pituitary cells actually secrete prolactin in cases of hyperprolactinemia with pituitary adenoma has recently been raised by some authors[65] who found a patient with Forbes-Albright syndrome in whom normal pituitary surrounding the adenoma was actually secreting most of the prolactin.

Surgery produced minimal discomfort in most patients. Average hospital stay was five days. Although nasal septal perforation caused minor irritation in a few patients,

their only complaint during workup and evaluation was of headache after pneumoencephalogram which, in a few instances, lasted from weeks to months.

The four patients with TSH hypersecretion had pituitary enlargement secondary to TSH cell hyperplasia or neoplasia from long-standing primary hypothyroidism.[50] Whether any of these hyperplastic pituitary glands will become autonomous and continue to grow, remains to be seen. In three patients, TSH levels measured to date have declined to normal, making this possibility unlikely, although one patient continues to have an elevated prolactin.

LH hypersecretion had been rarely reported.[18] Our patient with LH hypersecretion also secreted prolactin. She has not yet had surgery.

CONCLUSION

We feel that transsphenoidal pituitary adenectomy is a reasonable approach to all hypersecretory adenomata. Morbidity is low, and rate of cure, although not perfect, is good, especially if patients with large unresectable lesions are selected out.

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PITUITARY PANEL DISCUSSION

Rudi Ansbacher, MD	Chief, Department of Obstetrics and Gynecology, Letterman Army Medical Center, San Francisco, CA.
Loren I. Dolman, MD	Chief, Endocrine-Metabolic Ward, Letterman Army Medical Center, San Francisco, CA.
Jerry M. Earll, MD	Chief, Department of Medicine Walter Reed Army Medical Center, Washington, D.C.
William D. Odell, MD	Chairman, Departments of Medicine and Physiology, Harbor General Hospital, Torrance, CA, Professor of Medicine, UCLA Medical Center, Los Angeles, CA.
J. Blake Tyrrell, MD	Assistant Clinical Professor, Metabolic Research Unit, University of California Medical Center, San Francisco, CA.
Eckehart Wiedemann, MD	Assistant Clinical Professor, Donner Pavilion, Lawrence Berkeley Laboratory, University of California, Berkeley, CA.

Doctor: You mention that some women don't have galactorrhea, but amenorrhea only. What other clinical features does one look for before ordering a prolactin level in these patients?

Dr. Dolman: It depends on availability and cost-effectiveness of ordering a prolactin level on all the patients you see. If you see thousands of patients with amenorrhea, you might consider setting up your own assay. On the other hand, if you see only an occasional patient,

you might follow her for a while prior to obtaining the prolactin level. The prolactin assay is not that expensive compared to many other tests, and I'm obtaining it more frequently now on patients who have amenorrhea, where I can't find a specific cause. Of course, these patients need a good gynecological workup, including a pelvic examination by a competent physician, in addition to a good drug history, etc. Perhaps some day the prolactin assay will be a routine screening test for patients with amenorrhea.

Doctor: How much does it cost?

Dr. Dolman: Cost at Nichols Institute, which has one of the better prolactin assays, is about $30.00.

Doctor: As you know, Dr. Hammond at Duke University, who worked with hyperprolactinemia, estimates that 12% to 15% of women with menstrual aberrations have elevated prolactin. This ranges all the way from just dysfunctional luteal phase, to anovulatory cycles, to oligomenorrhea, to pure amenorrhea, to amenorrhea with galactorrhea. At this point, you almost have to draw a prolactin on every patient you see in your office!

Doctor: You mentioned that you do an average of three prolactin samples. If you were to get a false result with a single sample, which would be more likely, a falsely low in a person who has a prolactin-secreting tumor, or a falsely high in a normal person?

Dr. Dolman: Falsely high in a normal person, caused by a peak of episodic secretion. A pooled specimen tends to average out any episodic spikes.

Dr. Odell: May I make a couple of comments to try to put this in a different perspective? I think we have to proceed with some caution in the diagnosis and consideration of treatment in prolactin adenomas. It is interesting that only 10 years ago prolactin secretion as a cause of amenorrhea was considered extraordinarily rare, and the possibility of pituitary tumors secreting prolactin was considered unlikely. We would always try to express some fluid from the breast, and we would discuss what it meant. We've now taken three different areas of medicine and

married them into the current ability to diagnose a syndrome which probably is thousands of times more frequent than we then believed, so frequent that we now don't know what the syndrome means. The three different areas of medicine are: First, the advance ability to interpret a sellar tomogram. This wasn't possible 10 years ago. And clearly patients that had "normal" sellar tomograms 10 years ago can have their x-rays reviewed and interpreted as "an asymmetry to the left." In some patients, one didn't get tomograms, only a lateral view of the sella, and one didn't know the patient had a tumor. Many of these patients still have no problems 10 years later, even though now we can show they have an elevated PRL level. Second, the advent of microsurgery, which began in Paris, was moved to Montreal, and now is much over North America. And third, of course, is the development and refinement of the prolactin assay. Currently the statistics you mention from Dr. Charles Hammond have been amplified. At the pituitary conference that Dr. John Linfoot organized in San Francisco in 1978, a very large study from Japan stated that 18% of the patients presenting with amenorrhea had elevated PRL as the cause. We've thus gone from extraordinarily rare to 18% of the patients coming to the doctor's office. At the same time, when we do post-mortem examinations on patients who die of unrelated causes, we find 18% of these patients have a microadenoma in their pituitary. It never has produced a problem, and probably never will. Also, if we take serial tomograms on a normal population walking down the street, the best guess at the present time is 10% or 15% have the same kind of asymmetrical sella turcica, so we have now begun to marry several observations saying this patient has a disorder. Now, we frequently find microadenomas when the patients go to surgery, when they have amenorrhea and prolactin levels which are very high. But this is not the prolactin twice normal or a fluctuation from normal. So the aberrancy from a randomly obtained, slightly elevated prolactin isn't a terribly important differential in the treatment. Lastly, we do not know the natural history of these prolactin adenomas. Many patients may never have a problem except for oligomenorrhea, or amenorrhea, and, in some of the men, changes in libido and pontetia, etc. So we need to look at each patient and ask why he or she is coming to us, and why we should treat. And adding all that up, we consider whether surgery or leaving the patient alone is the best choice.

Doctor: Where are somatomedin levels available?

Dr. Wiedemann: I don't think they are commercially available at this time. But a number of laboratories using different assays are doing them and we're doing them fairly routinely at Donner Pavilion in Berkeley, both by bioassay and by radioreceptor assay.

Doctor: Does alcoholism in the absence of severe liver disease cause abnormal (growth hormone) response to the oral glucose tolerance test?

Dr. Wiedemann: I don't know. However, the growth hormone response to oral glucose is probably more complicated than it appears. A few years ago, I did glucose tolerance tests in approximately 35 normal subjects, ages 40 to 71, with a normal glucose tolerance. In these subjects, approximately one-third had growth hormone values higher at one hour than baseline. So a slight increase after one hour is probably normal in a significant percentage of the population. Abnormal results in the oral glucose tolerance tests are probably not as important as the results with the TRH test. The growth hormone rise (after TRH) in acromegalics is very characteristic, and does not occur in normal subjects.

Dr. Odell: What percentage of acromegalics have that kind of response?

Dr. Wiedemann: At least 90%.

Dr. Earll: I think, that in some series, it is as low as one-third.

Doctor: Which of the somatomedin family were you measuring in the acromegalic patients?

Dr. Wiedemann: This is a complicated problem. I did not specify somatomedin by any specific name because there is at this time no specific assay for any specific somatomedin. Every assay picks up several, if not all, of five different somatomedins that are presently recognized. And the results in these various assays are probably different, depending on the composition of the serum that you are assaying, so that there is a differential

response to the different somatomedins. It is impossible to say that you measure a specific somatomedin. The bioassay detects all of them. And the only one that is possibly specific (although that's not completely established yet) is a recent RIA for somatomedin C, developed in Van Wyk's lab. So I'd rather stick to just a descriptive term, bioassay for somatomedin, or receptor assay for somatomedin. And as I pointed out with the receptor assay, using human placental membranes, we found only one normal result in 50 untreated acromegalic subjects. With the bioassay, out of 133 patients, 72% had clearly elevated levels.

Doctor: In a patient in whom you have established the presence of hyperprolactinemia, and whose problem is amenorrhea, and you elect not to treat her, how do you follow her? Are you going to use tomograms? Suppose you do tomograms and find there's a microadenoma. How are you going to follow that patient?

Dr. Ansbacher: Dr. Odell brought out the key point, that we don't know the natural history of the disease. I personally would follow this woman with prolactin levels every six months and yearly sellar tomography. If there's no change to the "macro" adenoma status, she doesn't need anything. If the adenoma enlarges and interferes with other pituitary functions, she may need surgery; otherwise, there's no need to do anything.

Dr. Earll: I would think that after the first x-ray studies, with the prolactin level very low (meaning in the 20-30 ng/ml range) you would not need an x-ray every year. I would emphasize that if you count the x-ray dosage, you reach cataract levels of radiation. It's extremely important to check where your studies are being done, and that the eye is being protected with shields that will cut down a fair amount of the radiation.

Dr. Ansbacher: Jerry, I agree with you. There are tomographic cuts that can be done now that cut down on the exposure to the eye. If you know where the adenoma is, you can ask for specific cuts. That's what you should do if you notice a microadenoma in a specific place.

Dr. Earll: Most of the time you're not going to know.

Dr. Ansbacher: Well, if we have a microadenoma that we've already seen, and follow this patient serially, there is no reason to get the whole polytomography, only the serial cuts that you ask for. This cuts down the exposure.

Dr. Odell: I think it's difficult to know exactly what is the best thing to do at the moment. Dr. Earll has put his finger on an important problem. I've asked every radiology department head that I've been able to talk to in the last year or so, how many of them shield for cataracts, and I'm surprised to find that shielding is very rare. About 10 tomograms gets you to a cataract dose, as far as I can estimate. So it is wise to limit your tomograms to as few as possible, and your cuts to the area of interest at the moment. And if your prolactin really is 20 or 30, you might even reduce further the number of tomograms, because it's not too likely based on present data that you have an adenoma--the higher the prolactin, the more likely an adenoma is present. However, counteracting that is the excellent data that Dr. Hardy has collected, that the curability relates to the stage of the tumor. And if we miss and wait too long and get an invasive stage II tumor, then our cure rate is poor.

Doctor: What is the role for CT scanning pituitary tumors to look for suprasellar extension? How reliable do you find it?

Dr. Tyrrell: This is a question we've frequently discussed with Hans Newton, our neuroradiologist, asking, when can you show us the suprasellar region adequately enough to relieve us from doing a pneumoencephalogram? We did a serial study, particularly with patients with relatively small tumors, showing the CT as currently done here (UCSF) is inadequate to show mild degrees of suprasellar extension. If you have a patient who already has a visual field deficit, you'll see it on the CT. But for the patient who doesn't, you won't know. In a number of our patients with Cushing's disease, as an example, we did CTs. And of that group, we missed two with small suprasellar extension. There will be available newer high

resolution CT scanners, but it is still not clear whether they will show minimal suprasellar extension or even intrasellar lesions, i.e., tumors versus empty sella. We may know in the near future.

Dr. Wiedemann: Dr. Tyrrell has pointed out some of the problems with the CT scan. I also think it is quite clearly inferior to pneumoencephalography in terms of resolution and we would not rely on CT scans to delineate the optic chiasm. We frequently get CT scans from referring physicians, but the information we gain from them is quite limited.

Dr. Odell: We have developed an enormous experience in CT scanning. Up until three or four years ago, we would send out, under special arrangements, one to three patients for CT scans per week. We now have one scanner on 24-hour use, and it's becoming so common that it's routinely done before lumbar punctures if there's absolutely any question that there might be increased intracranial pressure. Perhaps this use is totally wrong. But we use the CT commonly in pituitary tumors, and I must say we've been very gratified. We have a high resolution machine with our neuroradiologist, Grant Hayashima, and have picked up many intrasellar tumors and many patients with empty sella syndromes. In general, our correlation with the pneumoencephalogram has been quite good for suprasellar extension. You can't generalize these findings to other institutions, however. And we're still learning. We now make more frequent cuts and are using contrast.

Doctor: In the post-menopausal woman with prolactin-secreting microadenoma whose main complaint is galactorrhea, how do you approach her treatment?

Dr. Dolman: One must decide if the patient is complaining about the galactorrhea because it is physically annoying, or she simply wants to know its cause. If it is a physical problem, bromocriptine (Parlodel®) therapy is a reasonable choice. This drug is a dopamine agonist which will lower prolactin levels in normal subjects and in most patients with hyperprolactinemia. I find more often than not the reason patients don't like galactorrhea is because they are concerned as to its cause, not because the

discharge is so bothersome. Some women may get mammary abscesses, and in them also bromocriptine therapy would be my choice.

Dr. Odell: And it also goes without saying that if they're on exogenous estrogen therapy, that may magnify the prolactin hypersecretion. Estrogen should be discontinued.

Doctor: I have one patient who has galactorrhea, and a normal prolactin level about 18. She insisted on trying bromocriptine despite the fact that it was not released by the FDA, except for hyperprolactinemic patients. It stopped her galactorrhea. Can you comment on that?

Dr. Odell: Was her growth hormone normal?

Doctor: Her growth hormone was normal as well as her T_4 and polytomography of the sella turcica.

Dr. Earll: Have you done any other studies such as a TRH stimulation?

Doctor: No, only an insulin tolerance test.

Dr. Odell: Bromocriptine has many other actions that we're just beginning to elucidate. It will suppress the growth hormone in some acromegalic patients quite effectively. It will remit amenorrhea not associated with hyperprolactinemia in some patients. It has central nervous system effects which do other things which we don't understand at the moment. We would probably have to conclude that we don't know why this lady has galactorrhea. But I will remind the audience that Dr. Vanderlaan's group at La Jolla has done excellent work with growth hormone, many different forms. Each is clearly distinguishable from the other, some of which have prolactin-like activity, and some which have somatotrophin-like activity without prolactin activity. They are measured with very different propensities by different antibodies. It is a big spectrum of structurally related proteins. The kind of patient you describe may become more common, and maybe she makes a substance that has prolactin-like activity, not measured in the current prolactin assays that you tested her in.

Dr. Dolman: In Franks' data from England and in a large German series, patients like yours have been treated with bromocriptine as a trial and many successes are noted. Dr. Odell has eloquently discussed various possibilities why this therapy might work. Bromocriptine will lower prolactin to essentially zero in normal individuals if enough is used in the correct way. Perhaps prolactin is permissive for galactorrhea in some patients without being elevated, and there is another abnormality accounting for the galactorrhea.

Doctor: Could Dr. Tyrrell comment on working up patients with Cushing's disease who are on Dilantin® and his experience with hydrocortisone suppression. We recently had a patient who had some clinical stigmata suggestive of Cushing's who was on Dilantin® for many years for a seizure disorder.

Dr. Tyrrell: There is a hydrocortisone suppression test, as you mention. However, we have little experience with it. What we have done prior to the publication of the paper on the hydrocortisone suppression test was to simply measure the 24-hour urine-free cortisol. In one patient who was on Dilantin®, who did in fact have Cushing's disease, markedly abnormal urine-free cortisol levels were obtained and we were able to simply rely on that. There has been a problem with virtually every drug and every test in some combination or another, and in certain circumstances where you can't take a patient off the drug, you are bound to work up the patient the best you can.

Dr. Dolman: The original work with the hydrocortisone suppression test came from the University of Utah and, while I was there, I had some experience with the test. I always found the results difficult to interpret. To summarize the test, what we are talking about is giving hydrocortisone rather than dexamethasone to suppress adrenal function (the hydrocortisone metabolism is affected less by Dilantin® than by dexamethasone). Since one cannot then measure cortisol levels, one measures another compound which should be suppressed, usually corticosterone. There are some problems with the corticosterone assay, even though Dr. Don West's lab at Utah had a very good one. The variability of the results was such that the test was still

difficult to interpret. None of those patients I tested turned out to have legitimate Cushing's disease.

Dr. Odell: Dr. Wolfson and I some years ago worked out careful dose-response curves between plasma ACTH and cortisol concentrations made constant in patients by carefully controlling the infusion rate of hydrocortisone. The normal individual has a dose-response curve that goes between about 0.1 and 20 micrograms per 100 ml. A patient with standard Cushing's disease is shifted over exactly four-fold, going from about 0.4 to somewhere between 90 and 100 micrograms per 100 ml. By controlling or by just looking at the cortisol concentration, and taking several samples of ACTH, one might determine which dose-response curve they are on. Patients with ectopic ACTH syndrome fall off the whole system, having high ACTH. So with reliable ACTH assays (remembering ACTH is a fragile hormone, with need to collect the blood sample on ice, etc.), you can get at this problem nicely. It's the *oral* test that is bothered by the Dilantin®, not intravenous testing.

Dr. Lttinger*: I think we have all noted hypoadrenalism after microadenoma removal for Cushing's disease. Several cases have been mentioned to me that have needed supramaximal levels of steroid postoperatively, just as we see with adrenalectomy. What is the explanation in terms of understanding the pathophysiologic mechanisms following surgery?

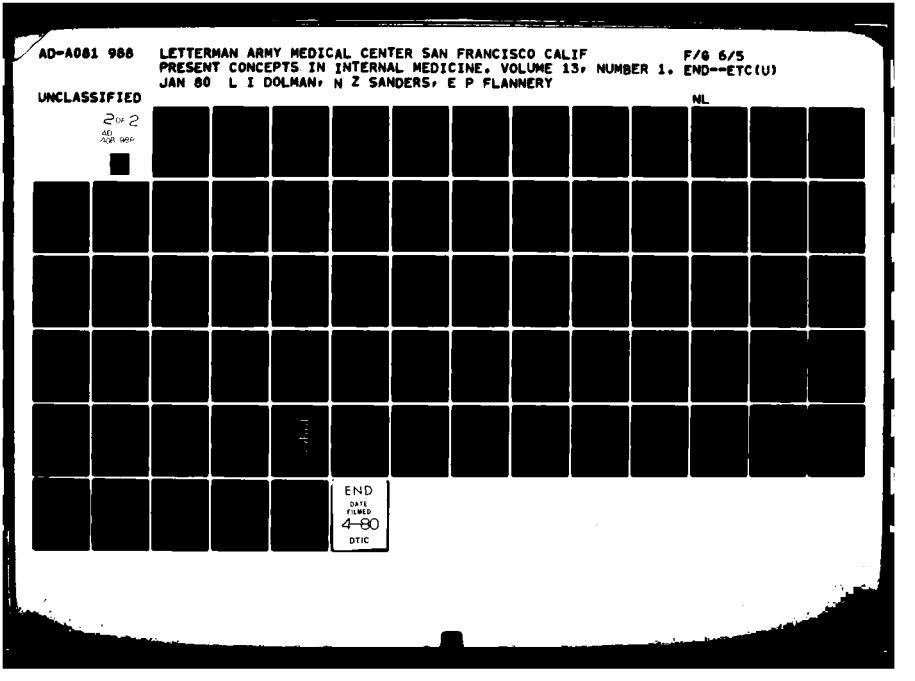
Dr. Tyrrell: I think that basically what it comes down to is that in a patient with Cushing's disease who has hypercortisolism, you send him to the operating room; and five days later you try to get him down to a normal replacement dose of hydrocortisone. It doesn't very often work, although we try it. We often have to back up again. It's what I call "steroid dependence" or "steroid withdrawal." I think you would see the same thing in a high proportion of patients who were on exogenous steroids if you tried to taper them so rapidly. That's the only adequate way I can explain it.

**Endocrinology Division, Kaiser-Permanente Medical Center, San Francisco, CA*

Dr. Ettinger: How do you explain the low adrenal function right after surgery? You made some measurements in two months, but others have seen seven earlier on low adrenal function.

Dr. Tyrrell: When you are covering the patient for the procedure, it is not clear now much you are actually suppressing him. What we saw after a successful operation in our patients was that the plasma cortisol levels were down by the following morning, and stayed down, sometimes unmeasurable at that point in time. Although the adrenal is hyperplastic and hyperfunctional, it is totally dependent on ACTH. If you remove the ACTH by dexamethasone or surgery, then the adrenal becomes rapidly nonfunctional. When we did serial ACTH stimulation tests we found that although in the first week the basal plasma cortisol had fallen to subnormal levels, the responsiveness to ACTH was still intact. As you tried at 10 days to several weeks, the adrenal gland actually became hyporesponsive. So I think it's that you have an adrenal that's totally dependent on ACTH. Hypoadrenalism results from lowering the endogenous ACTH too rapidly.

Dr. Dolman: Thank you for your participation. This concludes our Pituitary Panel, 1979. I wish to thank the panel members, and Nichols Institute for sponsoring us this evening.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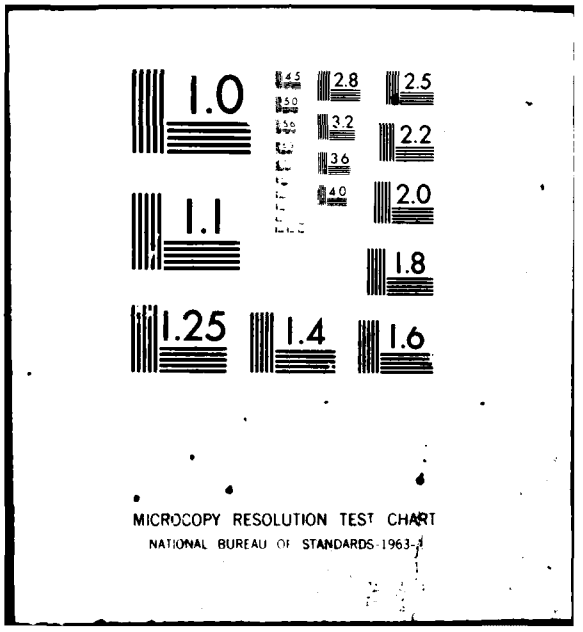
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VITAMIN D: THE SUNSHINE HORMONE

*Daniel D. Bikle, MD

Within the last 10 years, our knowledge about vitamin D has increased considerably. We have pushed vitamin D out of its traditional status as a vitamin to its new status as a steroid hormone.[1] This steroid-like hormone, acting in conjunction with parathyroid hormone, controls calcium and phosphate homeostasis by altering calcium and phosphate movement across its three principal target tissues: bone, kidney, and gut. Vitamin D itself is really a prohormone, since it must be metabolized in the body to a number of other compounds which mediate the biological effects. Understanding the metabolism of vitamin D and its effects on its target tissues has permitted a remarkable increase in our understanding of a variety of disorders in calcium and phosphate homeostasis. In this review, we will examine the metabolism of vitamin D, the effects of the metabolites on the three principal target tissues, and a number of clinical problems resulting from a disorder in either the metabolism or biologic effectiveness of the metabolites.

METABOLISM OF VITAMIN D

The advances in vitamin D research most applicable to clinical medicine today have elucidated the conversion of vitamin D to biologically active forms. First of all, vitamin D never would have become a vitamin had it not been for the industrial revolution. Under the influence of ultraviolet irradiation (sunlight), 7-dehydrocholesterol is converted to vitamin D in the skin. *Ergo,* the skin becomes an endocrine gland and vitamin D a hormone. Reduce exposure to sunlight, and man becomes dependent on dietary sources for vitamin D. Vitamin D becomes a vitamin. Regardless of

*From Letterman Army Institute of Research, San Francisco, CA

its origin from skin or diet, vitamin D must be metabolized further to become biologically active in less than pharmacologic concentrations. Hydroxylation of the side chain of vitamin D to 25 OHD occurs in the liver and, perhaps, in other organs like the kidney and intestine.[2,3] One important clinical aspect of this initial liver hydroxylation is that drugs like diphenylhydantoin (Dilantin®) and phenobarbital, which stimulate the microsomal mixed function oxidases in the liver, increase the conversion of vitamin D to biologically inactive metabolites.[4,5] Therefore, less 25 OHD is produced. Since a wide variety of drugs alter these microsomal enzyme systems, we will probably see more drugs associated with abnormalities in calcium and phosphate metabolism once we start looking for them.

Although 25 OHD is the main circulating form of the hormone, it is not the most potent. Rather, 25 OHD must be hydroxylated further to $1,25(OH)_2D$ by the mitochondria of kidney tubules.[6] This enzymatic reaction is similar to the steroid hydroxylations in adrenal mitochondria.[7] This similarity is further evidence that nature intended vitamin D to be a steroid hormone, not a vitamin. $1,25(OH)_2D$ is the fastest acting, most potent metabolite of vitamin D yet discovered.[8] As of today, it appears to be the final form of the hormone, at least in terms of regulating intestinal transport of calcium and phosphate. The evidence that $1,25(OH)_2D$ is the final hormone form for other vitamin D-regulated events in other target tissues such as bone, kidney, and muscle is less compelling. 25 OHD is metabolized to products other than $1,25(OH)_2D$, such as $24,25(OH)_2D$ and $25,26(OH)_2D$.[9] The production of $24,25(OH)_2D$ and $25,26(OH)_2D$ also takes place to a significant extent in the kidney.[10] However, these metabolites, unlike $1,25(OH)_2D$, are made elsewhere as well. The biologic functions of $24,25(OH)_2D$ and $25,26(OH)_2D$ are not clearly defined. Neither metabolite rivals $1,25(OH)_2D$ with respect to potency of action on the intestinal transport of calcium. Recent evidence suggests that $24,25(OH)_2D$ in conjunction with $1,25(OH)_2D$ may play a role in regulating parathyroid hormone secretion[11] and bone mineralization.[12] $24,25(OH)_2D$ may require hydroxylation to $1,24,25(OH)_3D$ before it is biologically active[13]--a step apparently utilizing the same kidney mitochondrial enzyme that converts 25 OHD to $1,25(OH)_2D$.[14]

De Luca and collaborators[15] have developed the concept that $1,25(OH)_2D$ and $24,25(OH)_2D$ are reciprocally related. In the animal with adequate vitamin D and calcium, $24,25(OH)_2D$ is made in preference to $1,25(OH)_2D$. In the animal deficient in vitamin D and/or calcium, $1,25(OH)_2D$ is produced. However, recent evidence from Stanbury's group[16] indicates that in primary hyperparathyroid patients, both metabolites are made in excessive amounts, suggesting that the reciprocal relationship between $1,25(OH)_2D$ and $24,25(OH)_2D$ is not universal. This discrepancy can be explained as follows. Parathyroid hormone (PTH), thyrocalcitonin (TCT), calcium and phosphate are all involved in the control of the 1α-hydroxylase and the 24-hydroxylase (the enzymes which produce $1,25(OH)_2D$ and $24,25(OH)_2D$, respectively) at two levels: synthesis of the enzymes (long-term effect), and modulation of their activities (acute effect).[15-23] The rachitic animal, regardless of the amount of calcium and phosphate in the diet, makes little or no $24,25(OH)_2D$.[19-20] The level of 1α-hydroxylase activity depends on the proportion of calcium and phosphate in the diet. In general, the lower the calcium and phosphate in the diet, the more $1,25(OH)_2D$ is produced.[20] The effects of PTH and TCT are difficult to separate from the effects of calcium and phosphate on enzyme synthesis although it appears these hormonal effects can be explained by their influence on calcium and phosphate levels in the blood. Administration of vitamin D along with adequate calcium abruptly reduces the level of 1α-hydroxylase activity and turns on 24 hydroxylase activity.[18] This switch from $1,25(OH)_2D$ production in the presence of vitamin D can be prevented by a diet low in calcium[13] or phosphate.[18] The change from $1,25(OH)_2D$ production to $24,25(OH)_2D$ production can be blocked by cycloheximide, and presumably is due to changes in enzyme synthesis. Acute control of 1α-hydroxylase activity is different.[17,20,21,23-25] These acute effects are not dependent on protein synthesis. PTH acutely stimulates and TCT inhibits $1,25(OH)_2D$ production by isolated renal tubules.[17] Calcium and phosphate exert a marked influence: high concentrations of calcium and phosphate inhibit 1α-hydroxylase activity, but the absence of calcium and phosphate is also inhibitory.[20] Maximal $1,25(OH)_2D$ production *in vitro* occurs at low but physiologic calcium and phosphate concentrations.[20,21] The acute control of the 24-hydroxylase by calcium and phosphate is similar,[24]

in that low but physiologic concentrations of calcium and phosphate stimulate 24,25$(OH)_2D_3$ production. In short, it appears that switching from 1,25$(OH)_2D$ production to 24,25$(OH)_2D$ production (long-term effect) requires time, because a new enzyme (24 hydroxylase) must be made. Vitamin D and high extracellular calcium and phosphate levels are required, conditions which block the synthesis of the 1α-hydroxylase. As such, 1,25$(OH)_2D$ and 24,25$(OH)_2D$ production are reciprocally related. However, both enzymes are acutely controlled in the same way, such that both enzymatic activities will respond in the same direction to acute change in intracellular calcium and phosphate levels. As such, the acute changes in 1,25$(OH)_2D$ and 24,25$(OH)_2D$ production are not reciprocally related. Therefore, PTH may stimulate both enzyme activities acutely but increase the amount of the 1α-hydroxylase relative to the 24-hydroxylase over the long term. The combined acute and long-term effects of PTH could result in elevations in both 1,25$(OH)_2D$ and 24,25$(OH)_2D$ levels in the blood. Increased sophistication in diagnosing and treating disorders in calcium and phosphate metabolism will come from a definitive understanding of the relationship between 1,25$(OH)_2D$ and 24,25$(OH)_2D$.

MECHANISM OF ACTION OF VITAMIN D

The mechanisms of action of the vitamin D metabolites are beginning to be understood. Vitamin D and its metabolites regulate calcium and phosphate flux across the epithelium of a number of tissues including intestine, bone, and kidney. The intestine has been most extensively studied. 1,25$(OH)_2D$ is the most potent metabolite affecting this tissue. Besides stimulating calcium and phosphate absorption, 1,25$(OH)_2D$ stimulates several enzymes including adenyl cyclase,[26] alkaline phosphatase (alk Pase),[27] Ca ATPase,[28] and RNA polymerase,[29] induces a calcium-binding protein ($CaBP_i$),[30] alters membrane lipid composition,[31] and enhances calcium uptake by mitochondria.[32] The question is whether any or all of these actions mediate the effect of 1,25$(OH)_2D$ on calcium and phosphate transport. 1,25$(OH)_2D$ acts like other steroid hormones. It binds to a specific cytosol receptor and is transferred to the nucleus.[33] It stimulates RNA polymerase[29] and *de novo* protein synthesis

(CaBP).[30] The problem is relating these events to calcium and phosphate transport. One can block CaBP production and alk Pase with cycloheximide (a protein synthesis inhibitor) without interfering with the stimulation of calcium transport by $1,25(OH)_2D$.[34] Furthermore, calcium transport is stimulated by $1,25(OH)_2D$ prior to CaBP production or alk Pase activity.[35] Our current understanding[36] of the mechanism(s) of action of $1,25(OH)_2D$ on the intestine is that $1,25(OH)_2D$ exerts its influence at several levels:

1) *de novo* induction of new proteins by a mechanism similar to other steroid hormones.[37] CaBP appears to be the only known protein induced *de novo* by $1,25(OH)_2D$.[38]

2) post-transcriptional stimulation of protein synthesis. Alk Pase seems to qualify for this mechanism.

3) direct effects not requiring protein synthesis. Stimulation of calcium movement into the cell across the brush border and into mitochondria qualifies.

We believe that c-AMP and calcium serve as "second messengers," mediating at least some of these effects.[39] CaBP may facilitate removal of calcium from the cell at the basolateral membrane in conjunction with a membrane-bound calcium pump (possibly the Ca ATPase). Alk Pase in the brush border may hydrolyze organic phosphates in the diet, freeing both calcium and phosphate for absorption. Much of this model requires further verification, but the basic concept that $1,25(OH)_2D$ works at multiple levels and not just by *de novo* protein synthesis is well established.

The action of vitamin D on bone is less clear. The assays currently in use measure a release of calcium from bone *in vivo* or *in vitro*. Such assays indicate that $1,25(OH)_2D$ is more potent than 25 OHD in releasing calcium from bone.[40-41] However, *in vitro* 25 OHD can mobilize calcium from bone without conversion to $1,25(OH)_2D$. Neither 25 OHD nor $1,25(OH)_2D$ require PTH for bone mobilization *in vitro*. Such assays shed little light on the mechanism by which these metabolites lead to bone mineralization, an effect seemingly opposite to bone mobilization. With our increasing understanding of the heterogeneity of bone cells, and our ability to separate these different bone cell

populations, this apparent paradox may soon be resolved and the molecular mechanisms better understood. It seems likely that the vitamin D metabolites regulate the function of the osteoclasts and osteocytes resorbing bone as well as the osteoblasts synthesizing new bone.

Whether vitamin D metabolites have a direct effect on renal handling of calcium and phosphate has been controversial. Recent evidence indicates that 25 OHD enhances reabsorption of calcium and phosphate in the absence of PTH.[42] $1,25(OH)_2D$ appears to be less active than 25 OHD, and $24,25(OH)_2D$ is inactive.[43] Vitamin D appears to be necessary for PTH to stimulate renal adenyl cyclase activity and the consequent phosphaturia.[44] Little is known about the molecular events underlying the effects of vitamin D on the kidney.

Vitamin D plays a role in muscle function.[45] Weakness is a prominent feature of the rachitic state. Calcium movement is abnormal in sarcoplasmic reticulum of rachitic muscle.[46] Birge and Haddad[47] noted that 25 OHD_3, but not vitamin D or $1,25(OH)_2D$, enhanced the ATP content and protein synthesis of muscle from rachitic animals. We should anticipate more research into the role of vitamin D on renal muscle function.

CLINICAL PROBLEMS SECONDARY TO DISORDERS IN VITAMIN D METABOLISM

With this overview of vitamin D metabolism and function in mind, we will examine how this knowledge may prove useful clinically. Disease involving vitamin D can be categorized into three areas: 1) Inadequate availability of vitamin D from the skin or diet, 2) abnormal metabolism of vitamin D to its biologically active metabolites, and 3) abnormal response of the target tissues to the metabolites.

1. *Inadequate availability.* If one is not exposed to enough sunshine or does not ingest enough vitamin D, rickets (osteomalacia in the adult) will develop. Such situations are rare in this country and are readily treated by adequate nutrition. Malabsorption of vitamin D or disruption of its

enterohepatic circulation secondary to cirrhosis, obstructive jaundice, pancreatic insufficiency, or other gastrointestinal disorders is a more common clinical problem and may be a serious component of the primary disease. The finding of a low 25 OHD level in the serum is the clue to the diagnosis. These patients can be treated with vitamin D supplementation and correction of the primary problem.

2. *Abnormal metabolism.* Disorders of vitamin D metabolism are most frequently found secondary to liver and renal disease. Biliary cirrhosis leads to osteomalacia that is not readily treated with vitamin D.[48] High doses of 25 OHD have proved more effective than vitamin D, consistent with the concept that the disorder in biliary cirrhosis involves inadequate conversion of vitamin D to 25 OHD.[49] End stage renal failure is a well-known cause of bone disease. Four factors are involved: Deficiency of $1,25(OH)_2D$, low serum calcium, elevated serum phosphate, and secondary hyperparathyroidism. An important problem is inadequate conversion of 25 OHD to $1,25(OH)_2D$ by the diseased kidney, although in mild renal failure $1,25(OH)_2D$ levels in blood may actually be increased concomitant with elevated PTH concentrations. Both vitamin D and 25 OHD have been used in treating this form of bone disease, but high doses are required. Hypercalcemic episodes occur and persist because of the long biological half lives of these compounds. Dihydrotachysterol (DHT) is more effective than vitamin D in renal failure because the steric configuration of the 3β-hydroxy group of DHT is similar to the 1α-hydroxy group of $1,25(OH)_2D$ such that it does not require the 1α-hydroxylation (the renal hydroxylation) to be active.[50,51] Recent results with $1,25(OH)_2D$ and its synthetic analog 1αOHD indicate that these compounds will become the treatments of choice in the near future.[52-55] $24,25(OH)_2D$ also may be deficient in renal failure but it remains to be seen whether $24,25(OH)_2D$ and $1,25(OH)_2D$ together will prove to be optimal therapy for the disordered calcium metabolism associated with renal disease. It must be emphasized that these potent vitamin D metabolites are not the panacea for treatment of renal osteodystrophy. The abnormal calcium and phosphate concentrations in the blood must be corrected with judicious use of calcium supplements and phosphate binders in an effort to reduce the secondary hyperparathyroidism and the risk of soft tissue calcification when

the vitamin D metabolites are administered. A primary defect in the renal 1α-hydroxylase system in the absence of renal disease appears to account for the rare autosomal recessive "vitamin D dependency" syndrome described by Scriver.[56] Drugs such as Dilantin® can lead to osteomalacia by abnormal metabolism of 25 OHD.[5] Part, if not all, of the complications of hyperparathyroidism and hypoparathyroidism can be related to alterations in $1,25(OH)_2D$ and $24,25(OH)_2D$ production. The current therapy of choice for primary hyperparathyroidism in symptomatic patients is surgical removal of the hyperplastic gland(s). It is conceivable that medical therapy with antagonists of $1,25(OH)_2D$ and/or $24,25(OH)_2D$ may lie ahead. Hypoparathyroid patients can be maintained successfully on 1αOHD or $1,25(OH)_2D$.[57-58]

3. *Abnormal response.* The third category includes those diseases resulting from an abnormal response of target tissues to normal amounts of the vitamin D metabolites. The hypercalcemia of sarcoidosis is associated with a hypersensitivity to vitamin D. The biochemical basis for this is not clear. Glucocorticoid therapy is effective in reducing the hypercalcemia.[59,60] Glucocorticoids antagonize the action of vitamin D on the intestine[61] and on bone,[62] but the exact mechanism is not defined. Although this antagonism is useful in the treatment of sarcoidosis, it is an undesirable side effect accompanying most other uses of glucocorticoids and accounts for the sometimes severe osteoporosis of Cushing's syndrome. Hopefully, vitamin D metabolites or analogs will prove useful in reducing the osteopenic effect of glucocorticoids without altering their anti-inflammatory action. Certain diseases may result from specific defects in response of cells to the vitamin D metabolites, but little or no evidence for this is currently available. X-linked hypophosphatemia, idiopathic hypercalciuria, idiopathic osteoporosis, and various poorly understood diseases may turn out to be such samples. However, we need to know much more about the mechanism of action of the vitamin D metabolites before these relationships become clear and appropriate treatment can be designed.

SUMMARY

In summary, we have reviewed briefly the metabolism and mechanism of action of vitamin D. This knowledge has

been gained almost entirely over the past 10 years and is still incomplete. We have discussed three main groups of diseases related to vitamin D: Inadequate bioavailability of vitamin D, defects in the conversion of vitamin D to its active metabolites, and defects in cellular response to the active metabolites. Appropriate treatment of several of these diseases has resulted from our increased understanding of vitamin D. One should anticipate further success in the future.

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SOMATOMEDIN AND OTHER GROWTH FACTORS

*Sam F. Hull, MD
*Robert H. Herman, MD, FACP

Somatomedin (Sm) is the term applied to growth hormone-dependent factors in the serum which promote growth and possess insulin-like activity. Using this definition, there would appear to be several or many somatomedins. In practicality, the term most often refers to the entity that was previously called "sulfation factor."

Salmon and Daughaday,[1] in their now classic study, demonstrated that growth hormone (GH) acts on cartilage by inducing a mediator substance which is measurable in serum and which they termed "sulfation factor" (SF). The *in vitro* incorporation of radio-labelled sulfate into cartilage from hypophysectomized rats was stimulated by serum from normal rats but much less so with serum from GH-deficient rats. Administration of GH to GH-deficient rats restored SF activity in their serum, whereas the *in vitro* addition of even large amounts of GH caused no change in the stimulatory activity. They concluded that serum contains a factor which is GH-dependent and mediates the growth activity of GH. Their bioassay for SF was useful clinically to detect GH-deficient states and acromegaly, but was relegated to use in research laboratories after the development of radio-immuno- and receptor assays which measured GH more directly.

BIOLOGICAL EFFECTS OF SOMATOMEDIN

Subsequently, it has been shown that SF exerts multiple biologic effects on cartilage, including the incorporation of thymidine into DNA, uridine into RNA, amino acids into protein and conversion of proline to hydroxyproline.[2-5] It is now apparent that normal serum contains an unknown number of GH-dependent factors that stimulate growth in a variety of tissues. All of these GH-dependent factors have

*From Letterman Army Institute of Research,
San Francisco, CA

one thing in common: They mimic the *in vivo* and *in vitro* effects of insulin. Although immunologically distinct from insulin, these factors stimulate glucose utilization by muscle and fat,[6,7] lipid synthesis[7] and inhibition of some adenyl cyclase-mediated reactions.[8] The term, "sulfation factor," is too constrictive and a more inclusive term, "somatomedin," has been suggested. "Somato" connotes its relationship to the somatotrophic hormone (GH) and "medin" indicates its mediary role.

The insulin-like activity of Sm requires more discussion. Somatomedin mimics the actions of insulin in all *in vitro* bioassays which measure insulin-like activity. When assayed by its ability to stimulate glucose oxidation in rat epididymal fat pads, radioimmunoreactive insulin accounts for only 7% of the total insulin-like activity in serum.[9] The remainder of the activity has been named non-suppressible insulin-like activity (NSILA, or NSILA-s/NSILA-P when referring to the activity found in the soluble or precipitated portion of acid-ethanol extractions obtained in Sm and NSILA purification). NSILA-s more recently has been called insulin-like growth factor (IGF).

The effects of insulin and Sm are basically identical with respect to growth and glucose metabolism. The major difference is in their relative potencies. At subsaturating concentrations, the actions of Sm and insulin are additive, suggesting that they act through a common mechanism.[10] In muscle, Sm stimulates membrane transport of sugars and amino acids and promotes the synthesis of protein and RNA.[3] The time course of these events is identical to that of insulin. A rational explanation for the insulin-like effects of Sm is provided by the demonstration that, in a variety of tissues, Sm is able to compete with ^{125}I-insulin for binding to the insulin receptor on the cell membrane.[9,11,12] While there probably are separate receptors, there is competition between Sm and insulin for those receptors. The effects of Sm on carbohydrate and fat metabolism occur at rather high doses. Conversely, the growth promoting effects of insulin are obtainable at dosages of insulin greater than those observed in physiological states.

Insulin is known to inhibit gluconeogenesis. During the past year, Judy O'Connor, Jerry Kropp, Mike Caldwell

and the author studied the effects of somatomedin A on gluconeogenesis in rat livers stimulated by glucagon. Gluconeogenesis was measured by determining the conversion of ^{14}C-alanine to ^{14}C-glucose in 24-hour fasted rat livers perfused with a medium containing 5.0 mM glucose, physiological levels of amino acids, washed human red blood cells, 1% bovine serum albumin and Krebs-bicarbonate buffer, pH 7.4. After a 20-minute baseline period, hormones (glucagon, somatomedin, glucagon plus somatomedin, or no hormone) were administered for four minutes at 20 to 40 minutes to achieve a total dose of $4X10^{-9}$M glucagon and/or 100μU somatomedin. Conversion of alanine to glucose was measured at 40 and 60 minutes and compared with the results obtained at 20 minutes, before hormones were given (Table 1).

TABLE 1

CONVERSION OF ALANINE TO GLUCOSE AFTER PERFUSION OF RAT LIVERS WITH GLUCAGON AND SOMATOMEDIN

	% increase at 40 min (±SEM)	% increase at 60 min (±SEM)
Glucagon	60 ± 10	64 ± 9
Somatomedin	32 ± 6	23 ± 5
Somatomedin + Glucagon	27 ± 6	23 ± 8
Buffer alone	20 ± 3	25 ± 5

These data indicate that Sm completely inhibits glucagon-stimulated gluconeogenesis, at remarkably low doses. The effect may help to explain why growth hormone improves nitrogen balance in some traumatized patients. In such patients, one would expect glucagon activity to be increased and the effect of glucagon is primarily catabolic. Thus, growth hormone, which stimulates Sm production, may reverse catabolism through the effect of its mediator, Sm. Such a conclusion is somewhat dangerous, in that the system we used is not entirely physiological. The effect of Sm in

this system may be magnified up to 100-fold, because the perfusate did not contain somatomedin carrier protein to which Sm is ordinarily 90+% bound.

NSILA may be of significance in some pathological states. Megysei et al[13] have developed a receptor assay for NSILA which measures markedly elevated levels of NSILA in some patients with hypoglycemia related to extrapancreatic, non-insulin producing tumors. Using standard bioassays for Sm or NSILA, some workers have measured elevated activity in serum and tumor extracts in similar patients.[14,15] Proof for the role of NSILA in the etiology of tumor-related hypoglycemia is far from conclusive.

MEASUREMENT OF SOMATOMEDIN

The measurement of Sm is most frequently accomplished using bioassays. With little modification, Daughaday's original bioassay is still useful.[16] Fragments of costal cartilage from hypophysectomized rats are incubated in the presence of $^{35}SO_4$, amino acid-containing buffer and various concentrations of serum. The subsequent incorporation of $^{35}SO_4$ into chondroitin sulfate is determined by scintillation counting, and Sm activity is expressed as nanomoles of $^{35}SO_4$ incorporated per 100 mg of cartilage per time of incubation. A linear regression line is found between the uptake of labelled sulfate into cartilage and the logarithm of the concentration of serum. Values obtained are compared to the activity of pooled normal serum which arbitrarily is given a value of one unit/ml. Other bioassays use chick embryo cartilages[17] or slices of costal cartilage from young pigs.[18] Since Sm also stimulates thymidine incorporation into DNA, labelled thymidine has been used for assay in a cell culture system using glial cells[19] as the target tissue. Several other bioassays are used, but complete descriptions are beyond the scope of this paper. An assay that measures insulin-like activity has been used in purification procedures, and, more recently, receptor assays and radioimmunoassays are being developed. Using virtually all of the assays mentioned, it is possible to clearly differentiate hypopituitary, normal and acromegalic serum.

ISOLATION AND PURIFICATION OF SOMATOMEDIN

Isolation, purification and synthesis of Sm have been slow because of the minute amounts of Sm in starting materials and because the bioassays used to monitor the purification are time-consuming, tedious and expensive. Serum is usually used because there appears to be no organ of storage; and, although many tissue extracts have some activity, none of the extracts has greater activity than serum. Somatomedin activity is remarkably stable and withstands freezing, thawing, lyophilization, acidification and boiling for up to one hour.[9] Somatomedin in plasma is bound to a large carrier protein (molecular weight approximately 70,000)[20] and is found mostly in the β-globulins. Acid-ethanol (AE) extraction of 8.66 kg of Cohn fraction IV (from 1,000 kg whole plasma) produced 38 gm of a protein material with Sm activity (10% to 20% recovery).[17] Further purification with G-75 Sephadex chromatography resulted in a single peak of activity with a molecular weight of approximately 6,000-8,000 (14% recovery of starting material and 20,000-fold purification). After some intermediate steps, a final purification was performed by high voltage paper electrophoresis at pH 6.5 (2 million-fold purification).

The use of electrofocusing techniques at various pH levels has led to the discovery of three different somatomedins. Uthne[6] has isolated Sm A, a neutral peptide of approximately 7,000 daltons which stimulates $^{35}SO_4$ uptake by chick cartilages and exhibits NSILA at all stages of purification. He also isolated Sm B, an acidic peptide of approximately 4,700 daltons, which stimulates thymidine incorporation into human glial cells but has almost no insulin-like activity. Van Wyk[7] isolated Sm C, a basic peptide of about 50 amino acid residues which stimulates sulfate and thymidine uptake into rat costal cartilages and has insulin-like activity. The isolation of these three different somatomedins suggests there may be a family of them in serum. It is possible that differences in the isolation procedures or monitoring techniques have created artifactual differences. Poffenbarger[21] has isolated an

NSILA substance with a molecular weight of approximately 70,000 by different methods. Whether this represents the parent material from which the somatomedins are derived or a protein-bound material is unclear.

OTHER GROWTH FACTORS

If one accepts the definition of Sm as being a substance in the serum that is responsive to growth hormone and has growth-promoting activity, then there are a number of substances that could be considered somatomedins. Pierson and Temin[22] have purified a small peptide from calf serum which stimulates fibroblast multiplication in tissue culture in the absence of serum. It is called multiplication stimulating activity (MSA). MSA stimulates sulfate uptake in rat cartilages, possesses insulin-like activity in bioassays and competes with insulin in receptor assays. MSA is generally accepted as an Sm.

Epidermal growth factor (EGF) is a peptide (approximately 6,000 daltons), isolated from mouse submaxillary glands, which accelerates opening of the eyelids and eruption of the incisors in mice.[23] It also stimulates epithelial proliferation in some organ cultures and stimulates DNA synthesis. Epidermal growth factor is stimulated by androgens, but its relationship to GH is not clarified.

Nerve growth factor (NGF) is a peptide from mouse submaxillary gland which is similar in structure to proinsulin. It stimulates differentiation of neural tissue and maintains the mature cells.[24] It was first identified as a soluble fraction produced by certain tumors. It mimics the anabolic actions of both insulin and Sm, and specific receptors for NGF are found in all tissues with sympathetic innervation. The number of NGF-receptors in any organ is proportional to the amount of sympathetic innervation. It is believed that developing nerves tend to follow blood vessel paths because vessels are rich in sympathetic innervation. As is the case with NSILA receptors, NGF receptors can cross-react with insulin receptors.

Erythropoietin is a peptide growth factor for red cells which, like EGF and NGF, is sensitive to androgens. It is

also sensitive to growth hormone. In hypopituitary states, erythropoietin is low. With GH therapy it increases, and it is high in acromegaly.

Robinson et al[25] have isolated a growth factor for granulocytes from human urine, called granulocyte colony stimulating factor (GCSF). GCSF is low in certain types of leukemia and returns to normal when therapy induces a remission.[26] GCSF is also low in patients with leucopenia related to Felty's syndrome and increases when therapy with lithium causes an increase in white blood cells.[27] The hormonal regulation of GCSF has not been elucidated.

Gospardowicz et al[28] recently isolated a growth factor from bovine brain and pituitary which causes metabolic events in fibroblasts similar to Sm B and C, MSA, EGF and NSILA-s. This brain growth factor (also called fibroblast growth factor) stimulates limb regeneration in frogs. Its importance to mammalian systems is not known and its relationship to other hormones has not been completely studied.

These are a few of the peptides on the ever-growing list of growth factors which seem to cause in their respective target tissue a set of metabolic reactions leading to growth.

CLINICAL IMPORTANCE OF SOMATOMEDIN

A. Pituitary Disease States and Normals

The level of Sm activity is fairly constant over a very narrow range in adulthood. Stuart and Lazarus,[29] using a bioassay, found the range to be from 0.85 to 1.11 U/ml in normal adults. Young children have levels below 0.6 U/ml that rise to normal by four years of age.[30] In seven children (ages four to 15 years) with growth failure due to GH deficiency, the levels ranged between 0.35 to 0.64 U/ml. After administration of GH, serum Sm activity increased to normal.[29] At the other end of the scale, patients with acromegaly demonstrate elevated Sm activity.[19,31,32] It is unexplained why a radioreceptor assay for NSILA-S devised at NIH failed to differentiate between acromegalic and normal serum.[13]

B. Relationship to Growth Rate and Size

A modest rise in plasma Sm activity has been reported with increasing age from birth to adulthood. Sm activity correlates better than GH with the growth spurt in adolescence. This is not surprising since GH release is episodic in nature whereas Sm levels are relatively constant, exhibiting no diurnal variation.[33]

Hall and Filipsson[34] recently attempted to correlate growth with Sm levels in a varied group of Scandinavian school children examined annually over a period of four to 16 years for their anthropomorphic development. Out of their study, an interesting concept of dental maturation developed. The eruption of permanent teeth was measured repeatedly. The chronological age (A) at which a reference point on the eruption scale was reached was used for analysis with other variables. Thus at age A, all children were of equal dental maturity. The function of 1/A represents the velocity of dental development. Serum Sm levels correlated well with 1/A ($p < 0.001$). The mean Sm level for children who were divided into short, medium or tall at a chronological age of eight years correlated positively with height. Also, the velocity of growth during the year preceding reference point A for dental maturation correlated positively with Sm activity. This observation has implications for determining eventual growth and bone age in some children.

C. Laron Dwarfism and Other Short Stature

There are several instances in which Sm levels correlate better with growth than GH levels. The most striking example of this phenomenon is found in the Laron dwarfs. In 1966, Laron[35] described dwarfism in some members of Jewish families in which there were low levels of Sm and increased levels of GH. Phenotypically, they were pituitary dwarfs. Treatment with GH failed to cause a rise in Sm or increase growth.[36] Subsequent studies have shown that their GH is active in receptor assays, which implies there is a defective receptor for GH resulting in failure to generate Sm.[37] There also appear to be situations in which there are normal

amounts of Sm and GH, but a peripheral resistance to Sm results in short stature. Normal levels of GH and Sm have been found in African pygmies.[38] In Turner's syndrome, growth failure is accompanied by normal GH levels and normal or elevated levels of Sm.[36] Daughaday[36] has proposed the following mechanisms for the Sm defect in Laron dwarfs and pygmies (Fig. 1).

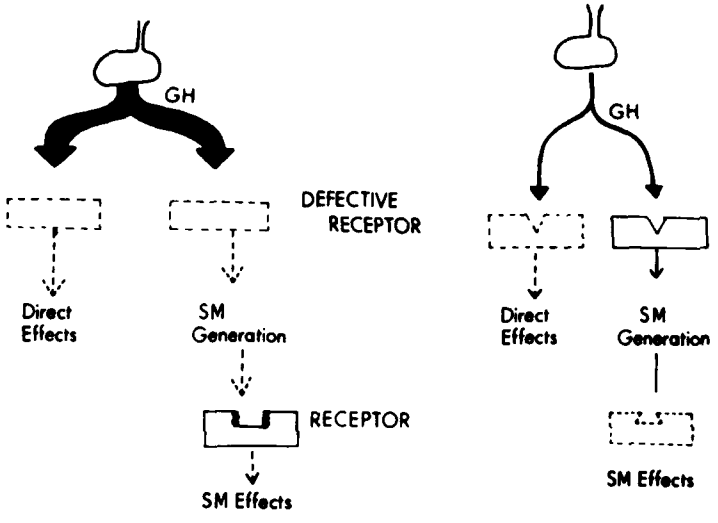

Figure 1. Left: Daughaday's explanation of the pathogenesis of Laron dwarfism. The tissue receptors for growth hormone (GH) are shown to be defective, both for somatomedin (Sm) generation and direct effects of GH. Right: The dffect in African pygmies is shown to be complex and involves both deficient direct responses to GH and decreased responses to somatomedin. (Reprinted with permission of the author and the publisher, Academic Press.)

D. Nutritional and Emotional Deprivation

It has recently become apparent that nutritional status may be another factor that effects a paradoxical relationship of GH to Sm. Elevated levels of GH and decreased Sm activity have been noted in children with kwashiorkor-type malnutrition and stunted growth,[39] and in patients with anorexia nervosa.[13] Phillips and Young[40]

have shown that rats fasted for 24 hours begin to have lowered Sm activity and by 72 hours reach hypopituitary levels. There are suggestions that a similar phenomenon occurs in humans. Emotionally deprived children may also have decreased levels of Sm and stunted growth.[41]

E. Diabetes Mellitus

Juvenile diabetes mellitus is a disease associated with stunted growth and decreased nutrient supply at the cellular level, a situtation qualitatively similar to fasting. In insulin-treated diabetics, Sm levels are normal. Phillips[42] has created diabetes in rats by treatment with streptozotocin. Within 48 hours, Sm activity was in the hypopituitary range. Not only was Sm low, but also serum from the diabetic rats appeared to have an inhibitor of Sm. Increasing concentrations of diabetic serum produced negative dose response curves. Mixing diabetic serum with normal rat serum produced lower Sm activity than a mixture of normal rat serum and hypophysectomized rat serum. Both of these observations suggest the presence of an inhibitor. Treatment with excessive GH did not alter the Sm activity or remove the inhibitor. Treatment with insulin, on the other hand, caused Sm activity to increase and the inhibitor to disappear within 24 hours. Although the presence of an inhibitor has been noted by several other investigators,[43] it has been more of a nuisance than a subject of research. However, the inhibitor may be an important physiological mechanism whereby the organism can limit anabolic events under conditions of restricted nutrient intake or utilization, thereby conserving substrate for more vital processes. The inhibitor is poorly defined to date, but is known to be non-dialyzable, heat labile (60°C for 1 hour), and is inactivated by trypsin.[43] Other materials which may produce inhibition are increased free fatty acids and glucocorticoids.

From these observations one could postulate that Sm is, in effect, a nutritional marker, i.e., when the mileu is appropriate for growth, Sm activity is normal. When almost any essential requirement for growth is missing, Sm activity is decreased.

Some studies performed at the Letterman Army Institute of Research may support this idea. Dr. Daniel Bikle studied

calcium and vitamin D metabolism in a group of patients with metabolic bone disease. During the course of the experiments, patients were on a calcium-deficient diet (100 mg/d) for one week and received an identical diet during a second week, except that calcium gluconate tablets were given to raise calcium intake to a normal level (1000 mg/d). We studied 10 patients (Table 2) and measured Sm A activity with a radioreceptor assay on fasting serum collected after each week. Somatomedin A activity was depressed after the low calcium diet in nine of 11 studies (Figs. 2 and 3).

TABLE 2

CLINICAL DATA ON PATIENTS STUDIED

			100 Mg Calcium Diet	1000 Mg Calcium Diet	
	Age Years	Sex	SM U/ml	SM U/ml	Diagnosis
1	59	F	0.72	1.13	Senile osteoporosis
2*	69	F	1.22	1.42	Senile osteoporosis, osteomalacia, same patient as 3
3*	70	F	1.13	1.59	Senile osteoporosis after 1 yr. treatment with Vitamin D
4	66	F	0.84	1.18	Senile osteoporosis
5†	41	M	0.99	0.91	Idiopathic osteoporosis of 10 yrs. duration
6	22	F	0.87	1.97	Diabetes melitus, idopathic stress fractures
7	25	F	1.58	2.09	Benign congenital osteopetrosis
8	65	M	2.02	0.79	Paget's disease, primarily cranial involvement
9	73	F	0.86	1.31	Hyperparathyroidism
10	69	F	0.48	0.82	Hyperparathyroidism
11	80	F	0.91	1.33	Hyperparathyroidism

* Patient 2 and 3 are the same patient, studied on 2 occasions

† The somatomedin values are significantly different for all patients except number 5 because of intra-assay variability.

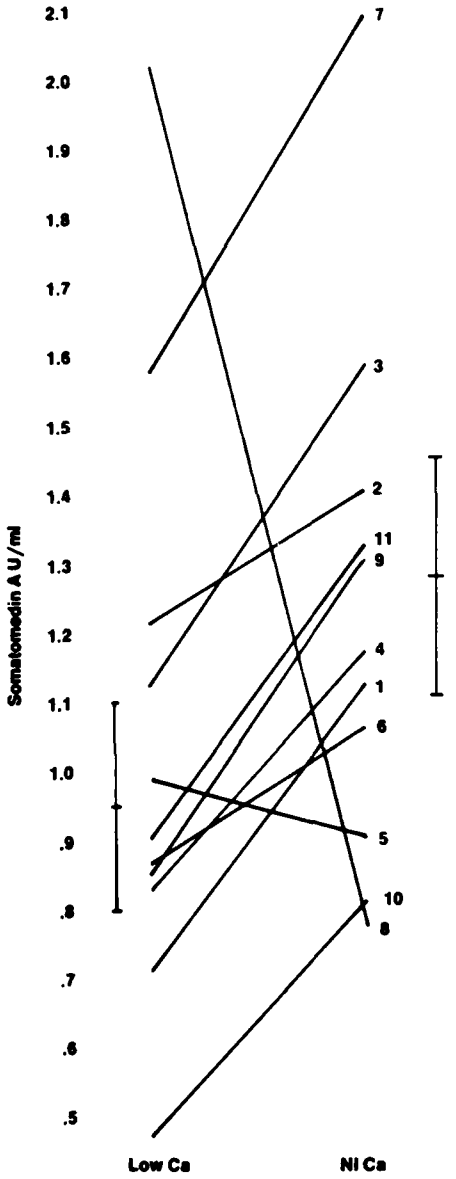

Figure 2. Serum Sm A activity (U/ml) following consumption of a low calcium diet (100 mg/d) and a normal calcium diet (1000 mg/d) for 1 week. The mean (± Std. Dev.) values are significantly different ($p < 0.0001$) with patient 8 excluded. The number identifies patients.

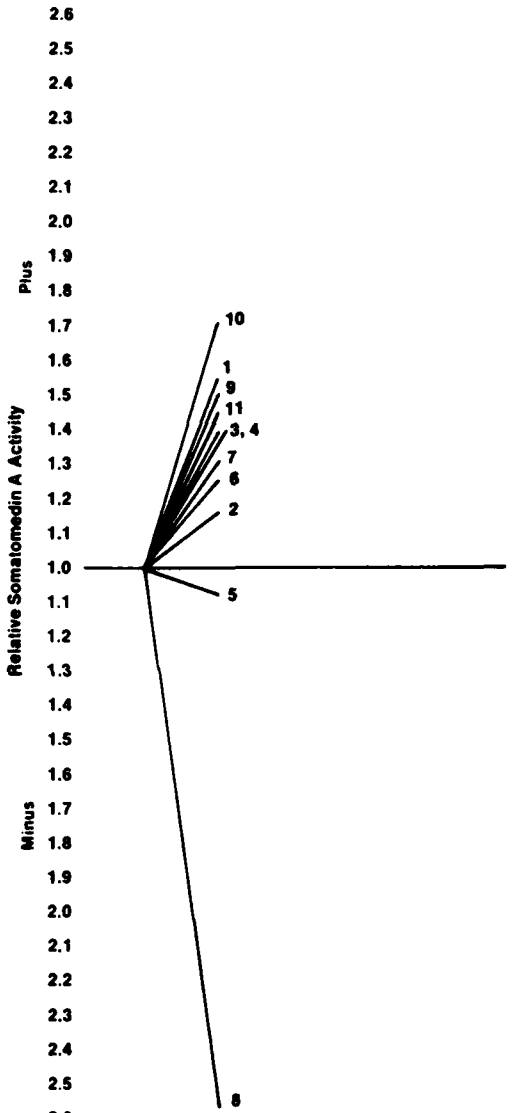

Figure 3. Relative change in Sm A activity with values corrected to 1.0 U/ml during the low calcium diet. The change observed after consuming a normal calcium diet is significantly positive by the sign test ($p < 0.01$). The number identifies patients.

Although we did not study a normal population, the diversity of clinical conditions leads us to believe that a depression of Sm A activity is the expected response to dietary calcium deprivation. The exceptions in these experiments were two males with idiopathic osteoporosis and Paget's disease. If one accepts the original observations that Sm controls bone growth, it would be reasonable to postulate that disordered regulation of Sm activity might lead to diseases of bone remodeling or uncontrolled growth. These studies suggest the need for further studies to determine if disorders of Sm production are involved in the pathogenesis of either idiopathic osteoporosis or Paget's disease.

F. Renal Disease

Another disease in which GH and Sm do not necessarily correlate well is renal failure. In renal failure, GH is frequently elevated but Sm activity as measured by bioassays is depressed. Following dialysis or renal transplantation, Sm returns toward normal.[29,44] The depressed activity is thought to be due to an inhibitor, because radioreceptor assays, which are not affected by the inhibitor, reveal markedly elevated Sm activity. The relationship of decreased Sm activity to the bone disease of renal failure has not been examined.

G. Estrogen Therapy

Estrogens exert some therapeutic effect in patients with acromegaly.[45] It is debated whether this is due to inhibition of GH release or a peripheral action leading to diminished production of Sm. The observation that estrogen treatment of acromegalics decreases Sm activity does not favor either hypothesis. Since it has been shown that giving estrogen to GH-treated pituitary dwarfs decreases Sm, and since estrogen has no *in vitro* effect on Sm, it can be concluded that estrogen diminishes production of Sm.[46] It is highly speculative, but if Sm acts as a negative feedback material on GH secretion, and there is some evidence that it may,[47] then an understanding of Sm may lead to an explanation of why females are generally shorter than males. It is interesting that adult females have slightly higher GH levels than males.

H. Hepatic Disease

In a group of seven cirrhotic patients, Stuart and Lazarus[29] reported reduced Sm levels (mean 0.35 ± 0.07 U/ml), despite significantly elevated serum GH levels.[29] McConaghey and Sledge[48] showed that rat livers perfused with GH produced Sm. Other studies have shown that partial hepatectomy reduces serum Sm and, as the liver regenerates, Sm increases.[49] Hypophysectomized and hepatectomized rats are unable to make Sm following GH administraton. Hypophysectomized rats with intact livers produce normal levels of Sm following GH administration. These and other observations in man and animals suggest that the liver is the major site of Sm production. There is less convincing evidence that Sm is produced by kidney and muscle. Whether the lower levels in cirrhosis are a function of cellular damage remains to be clarified and the clinical significance of lower Sm is not known.

I. Pregnancy

NSILA was elevated in five of six pregnant patients when measured by a receptor assay.[13] NSILA in cord blood was also elevated in two samples from cord blood. The significance of increased NSILA in pregnancy is unknown.

J. Exercise

Glucose utilization proceeds at an increased rate during exercise, despite the fact that insulin secretion diminishes. Since GH is secreted during exercise, one might expect that NSILA would be produced and could account for the glucose utilization. Stuart and Lazarus[29] measured Sm during exercise and found no significant change in the circulating levels.

K. An Unexplained Phenomenon

The numerous clinical situations that are characterized by paradoxical relationship between Sm and GH raise the

possibility that Sm is not totally dependent upon GH. Further evidence for this comes from an unusual experiment. Subcutaneous implantation of the parasitic tapeworm, Spirometra mansonoides, into hypophysectomized rats caused growth for four to six weeks.[50] Somatomedin was elevated in the rats and GH levels did not increase. Serum from infected rats caused growth in non-infected hypophysectomized rats. The stimulatory effect was lost after several weeks (at which time Sm also decreased), but it seems that factors other than GH are capable of stimulating Sm production.

Tremendous amounts of information regarding Sm have become available since the original description of Sulfation Factor in 1957. Already the knowledge has contributed to the understanding of some clinical conditions. Somatomedin is a hormone in its own right and further work will undoubtedly lead to discoveries in diagnosis or treatment of disease states and understanding of growth processes.

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NOTE: References 9 and 29 are well-written reviews that provide fairly up-to-date information on Sm. Another excellent review (not referenced) is Van Wyk JJ, Underwood LE: Relation between growth hormone and somatomedin. Annu Rev Med 26:427-441, 1975.

USE OF THE COMPUTER IN THE MANAGEMENT AND STUDY OF DIABETES MELLITUS

*James K. Schmitt, MD
*Joel R. Poole, MD
*Thomas A. Schultz, MD, FACP
*Stephen B. Lewis, MD, FACP

Diabetes mellitus has been recognized as a disease for thousands of years, and common modalities for its treatment have been available for decades. Yet, much controversy remains as to the etiology of the long-term complications of this disease and the most effective means of preventing them.[1-3] With development of the radioimmunoassay for insulin, it has become apparent that diabetes mellitus is not a single disease entity, but rather may be the manifestation of a spectrum of insulin-deficient and insulin-resistant states.[4] The lack of agreement among the medical community as to the efficacy of control of blood glucose in preventing long-term complications of this disease, coupled with studies such as the University Group Diabetes Program,[5] have further clouded the issue of how best to treat it in its different forms. As a result, competent physicians vary widely in their approach to the individual diabetic patient. In addition to lack of a universally accepted therapeutic plan, research in diabetes is often hampered by difficulty in retrieving data from charts that are sometimes illegible and disorganized.

With the development of modern computer technology and high-speed printers, the above difficulties can be ameliorated.[6-8] Over the past 2-1/2 years, we have employed the computer as a means of data storage and retrieval for our diabetic population. We are now using the computer to

*From the Clinical Investigation Center, Naval Regional Medical Center, Oakland, CA

implement treatment algorithms and to aid us in diabetes research.

A 64K mini-computer with two 80-million byte drives serves as the central processing unit. Data are inserted and retrieved at three video display terminals, one of which is located at the Diabetes Clinic of our hospital. Stored data are transferred to typed copy by a high-speed printer. When appropriate symbols and code numbers are typed, the computer prints out the patient's entire clinical record. Various options are available by which selected sections (such as laboratory data) on one or any number of patients can be extracted from the computer's memory bank.

PATIENT POPULATION

Approximately 1,350 diabetic patients, primarily adults, are treated annually at the Naval Regional Medical Center, Oakland, California. Although the preponderance of diabetes is of the maturity-onset variety, juvenile diabetes, diabetes of pregnancy, and other less common forms of diabetes are also represented.

METHODS

After informed consent has been obtained, the patient is scheduled for an initial visit to the Diabetes Clinic. At this time, a history sheet is completed with the aid of paramedical personnel. This information is then fed into the computer and a computer printout produced. The physician then reviews the printout and performs a physical examination. An initial assessment as to the type of diabetes, e.g., insulinopenic or obesity-related, is made. A standard laboratory profile including hemoglobin Al (expressed as per cent total hemoglobin)* triglycerides and cholesterol**, and lipoprotein fractions[9] is obtained.

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* Hemoblobin Al = HGB Ala + HGBAlb + HGB Alc (Bio-Rad Laboratories, Richmond, CA)

** Autoanalyzer II (Technicon Instruments Corp. Tarrytown, N.Y.).

Two weeks later, the patient is seen in the Diabetes Clinic by a physician. The computer printout has become a part of the permanent medical record and includes history, physical, and initial laboratory data. Based on these results, the physician begins an appropriate treatment regimen. Two weeks before each clinic visit, the patient completes an "Interim Questionnaire" which summarizes his physical status since his last clinic visit. The completed form is mailed to the Diabetes Clinic and the data are entered into the computer. When the patient again sees the physician, these data have already become part of the medical record and can be quickly scanned for any recent problems.

We are in the process of applying computer algorithms to the treatment of our diabetic patients. Based on the criteria of severity of diabetes, age, degree of complications, and coexisting medical problems, an initial treatment regimen is assigned by the computer. Whether or not the treatment dictated by algorithmic logic is changed at a later date depends on the response to therapy. Figure 1 illustrates a typical treatment algorithm for diabetes mellitus. When clinical studies are performed, the patient may be assigned randomly (based on his Social Security number) to one of several treatment regimens (Fig. 2).

Although the majority of man-hours involved in this system come from paramedical personnel, the computer printouts are regularly reviewed by a physician. When certain conditions are present, e.g., when blood glucose is dangerously high, the algorithm calls for direct consultation by the physician.

RESEARCH

The computer is a powerful tool for the study of chronic multi-faceted diseases such as diabetes mellitus. Besides implementing treatment algorithms as described above, the computer provides a method for retrieval of data from a large number of records in a short period of time. We recently used our computerized data base to answer two fundamental questions: 1) Is the patient's assessment of his diabetic control an accurate reflection of his control

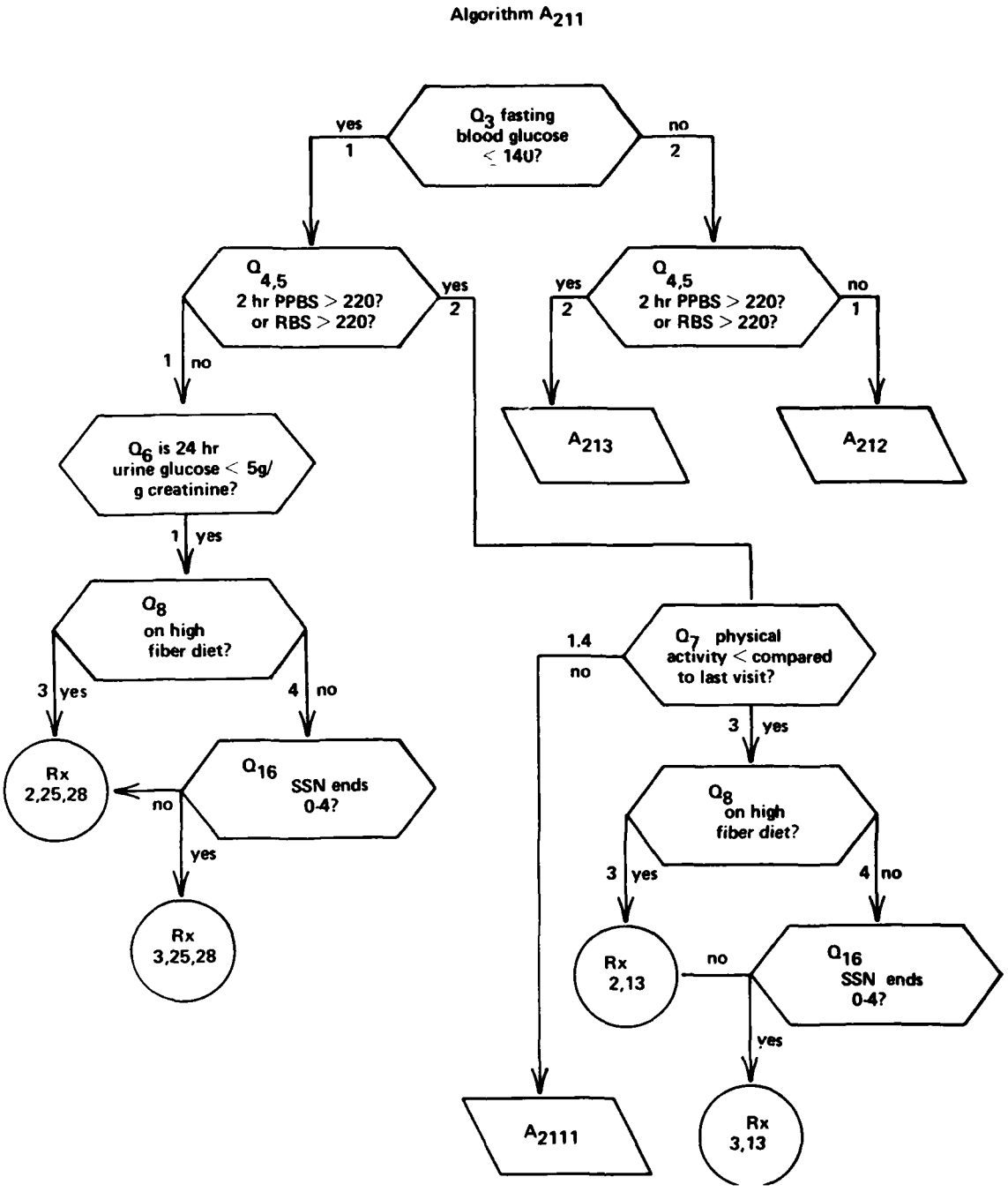

Figure 1. A typical treatment algorithm for computer management of diabetes mellitus.

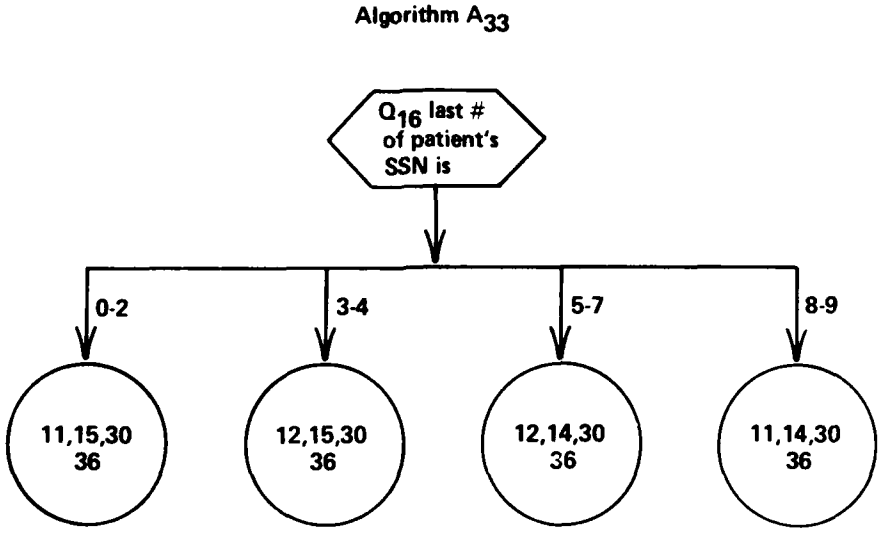

Figure 2. Assignment of patient to treatment regimen based on Social Security Number.

as determined by hemoglobin A1 level? 2) Is there a correlation between HBA1 and cholesterol in the lipoprotein subfractions HDL, LDL and VLDL?

Previous work[10] has suggested a good correlation between several parameters, including fasting and peak plasma glucose and HBA1. In the initial history, our patients evaluated their control during the preceding six months by estimating the percentage of their urine tests that were negative for glucose. From these data we classified the patients into three groups and compared mean hemoglobin A1 levels in each group (Table I). Thus, the conclusion that degree of diabetic control is reflected in the urine test has a certain degree of validity. Even in "good control group" (negative urine tests at least 75% of time), however, the mean hemoglobin A1 was significantly elevated above the normal range (6% to 9%), and the mean level was not significantly different between the "fair control" and "poor control" groups.

TABLE 1

HEMOGLOBIN Al LEVELS (% OF TOTAL HEMOGLOBIN) IN DIABETIC SUBGROUPS CLASSIFIED BY URINE TEST RESULTS REPORTED OVER PRECEDING 6 MONTHS

% Urines Negative	76-100	50-75	< 50
HBAl (Mean ±SEM)	11.5 ±.35 (a)	13.8 ± 1.18 (b)	14.9 ± 1.2 (c)

Level of significance: a versus b $p < 0.01$
a versus c $p < 0.005$
b versus c p not significant

It is well known that diabetics suffer from an increased incidence of cardiovascular disease.[11] Various studies indicate elevated serum cholesterol and triglyceride levels in diabetic patients,[12,13] and one study has shown a negative correlation between HBAl and HDL cholesterol.[14] We studied our patient population for a possible correlation between HBAl and cholesterol in the lipoprotein subfractions HDL, LDL, and VLDL. These data were extracted from the computer and standard linear regression analysis applied. Results are summarized in Table 2.

TABLE 2

CORRELATION BETWEEN HEMOGLOBIN Al (% TOTAL HEMOGLOBIN) AND HDL, LDL, AND VLDL CHOLESTEROL IN 66 DIABETIC PATIENTS

	HBAl versus HDL	HBAl versus LDL	HGBAl versus VLDL
Coefficient of correlation	r = -.30	r = .27	r = .31
Level of significance	$p < .05$	$p < .05$	$p < .01$

The negative correlation between HBAl and HDL agrees with earlier work,[14] and indeed the correlation coefficients are identical (r=-.3). In addition, there is a significant positive correlation between HBAl and LDL (r=.27) and VLDL

(r=.31). This information indicates a definite relationship between the various lipoprotein subclasses and the severity of the diabetes. To our knowledge, this is the first time it has been shown that degree of glucose control parallels levels of two major lipoprotein subclasses that are predictive of risk of myocardial infarction.[15] Longitudinal studies are now in progress to determine if reduction of HBA1 by treatment is associated with corresponding reductions in LDL and VLDL, and increase in HDL.

CONCLUSION

The computer provides a method for storage and rapid retrieval of data obtained from a large diabetic population. Time saved in data processing and decreased physician time required for an individual patient visit compensate for the cost of the computer hardware and personnel. This system is, therefore, cost-effective.

The ease of recovery of data in printout form makes the computer an ideal tool for research. In the near future, the computer will be programmed to directly analyze data by mathematical formulae (such as regression equations). It is now possible to generate statistics, literally overnight, that previously would have required months to obtain and analyze.

The use of algorithms in patient management is not really a new concept. In evaluating the patient, the physician goes through a series of logical steps based on information generated in the previous step. For example, if the patient has persistent glycosuria before dinner, the logical step is to increase the morning intermediate-acting insulin. The computer algorithm merely formalizes this operation.

The vast majority of our patients are enthusiastic about having their medical data computerized. When patients are shown an orderly printout of their medical information, the advantages of the computer in their medical care become readily apparent to them.

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STUDIES OF THE TROPHIC PROPERTIES OF GASTRIN ON THE GUT

*Robert E. Morris, MD
*William Rodkey, DVM
*Donald G. Griffin, DVM
*Robert H. Herman, MD, FACP

Gastrin is the gastrointestinal hormone produced and secreted by the G-cell of the gastric antrum. Some G-cells are also found in the duodenum, and a few in the jejunum. The two major forms of gastrin are a 34 amino acid chain, big gastrin (G-34) and a small 17 carbon chain, little gastrin (G-17). Both exist in a sulfated and unsulfated form.[1] G-34 is probably a precursor of G-17, since tryptic digestion of G-34 releases G-17 and an inactive NH_2 - terminal fragment, (NT-G34) G-cells, also contain equal molar concentrations of G-17 and NT-G34.[2]

Both big and little gastrin are biologically active but little gastrin has five times the acid-producing potency of big gastrin. Pentagastrin, the first five amino acids of the COOH terminal end of G-17 and G-34, is also active but to a lesser extent than G-17. Pentagastrin is the commercially available synthetic drug used in diagnostic medicine.[1]

Big and little gastrin are principally degraded in the kidney where the molecules are apparently metabolized since there is no gastrin found in the urine. The half life of little gastrin and big gastrin is three and 15 minutes, respectively. The intestine also plays a role in clearing gastrin, whereas the liver clears the small pentagastrin molecules.[1,3]

The release of gastrin from the G-cell is triggered by direct application of protein or certain chemicals onto the G-cell or by neuronal stimulation via the dorsal portion of the vagus.[4] In dogs, the neuronal stimulation can be mediated by sham feeding or gastric distension and can be blocked by atropine.[1] In man, gastric distension does not cause a rise in serum gastrin, whereas sham feeding does.[1] Protein-induced gastrin secretion is not blocked

*From Letterman Army Institute of Research,
San Francisco, CA

by atropine in dogs although it blocks acid secretion. The application of a local anesthetic to the mucosa preceding a protein stimulus blocks gastrin secretion and thus blocks acid secretion.[4] This effect supports the presence of two releasing mechanisms in the G-cell. One, the neuronal, has already been discussed. The other mechanism involves direct stimulation of G-cell itself without requiring any neuronal connection.

Gastrin has a variety of functions. Chief among them is stimulation of acid secretion by the parietal cells and pepsin secretion by the chief cells. When the pH of the stomach contents rises, gastrin is released which, in turn, causes acid secretion and a fall in pH with subsequent shut-off of gastrin secretion. Protein-containing foods directly stimulate G-cells, while other foods by virtue of their alkalinizing properties stimulate gastrin-release.[1]

Gastrin may also contribute to the maintenance of lower esophageal sphincter tone.[5] *In vitro* studies of the colon have shown gastrin to increase the net sodium transport across the mucosa into the lumen. This change in direction of sodium flow may account in part for the diarrhea present in Zollinger-Ellison syndrome.[6]

An intriguing effect of gastrin is its trophic effects on the stomach and other digestive organs. These effects include increased DNA synthesis as measured with ^{3}H thymidine,[7,8] increased protein synthesis as measured with ^{14}C leucine in the stomach and duodenum,[9,10] and maintenance of the endothelial character of gastric mucosa in tissue culture.[11,12] Mayston and Barrowman[13] demonstrated that post-hypophysectomy pancreatic atrophy in rats could be prevented by pentagastrin administration. The clinical significance of gastrin in maintaining intestinal function is incompletely understood. This could be clarified in part by studying the effects of experimentally produced gastrin deficiency and exogenous replacement of gastrin.

Most studies concerning gastrin effects on the rat intestine have been done in rats that have an intact gastrin-producing system. The effect of gastrin in a gastrin-deficient rat, with and without gastrointestinal stress, has not been studied in detail. Johnson and Chandler[14] did report an overall increase in gut DNA and RNA in gastrin

deficient rats created by antrectomy and treated with exogenous pentagastrin when compared with gastrin-deficient control rats. However, they did not measure the level of gastrin in the rat. Oscarson et al[15] reported that the degree of post-resectional bowel hyperplasia was independent of the endogenous levels of gastrin which were varied using antrectomy in the rat. Their group did measure gastrin levels three weeks after surgery and found the antrectomized rats to have a very low level of gastrin compared with controls. Further study of the effect of antrectomy on endogenous gastrin levels is needed to determine if an animal can be surgically rendered gastrin-deficient.

Starvation can also induce a low level of endogenous gastrin. Lichtenberger et al[16] showed that starvation in the rat decreases serum gastrin levels in the intact animal. Starvation also results in decreased villus height and crypt depth,[17] as well as decreased DNA and RNA in the entire small intestine.[16] Some studies have shown brush border enzymes from the gut [18-20] to decrease, not change,[21] or increase[22,23] measured in the entire small intestine. Lichtenberger et al[16] showed an increase in levels of maltase and lactase in the gut of starved intact rats. They also showed partial reversal of the above effects of starvation in normal rats by using exogenous pentagastrin during the period of starvation. However, they did not localize the areas of change; instead, they measured changes in the intestine as a whole. Thus, the role gastrin plays in these enzyme changes is not fully determined.

The postoperative period following repair of traumatic bowel injury always includes a degree of fasting. In antrectomized soldiers who have undergone extensive resection of bowel, the gastrin deficiency and starvation may be the two necessary coexisting factors leading to atrophy of the remaining bowel. Further work is necessary to compare the effects of starvation alone, and the combined effects of starvation and surgically induced gastrin deficiency.

The principal source of endogenous gastrin in animals is the antrum. There is some gastrin in the intestine; the amount varies, depending on the species of animal. In humans, there is substantial duodenal gastrin; in dogs there

is less.[24] In rats, the duodenal concentration of gastrin is stated to be from 500- to 100-fold less than the antral concentration.[25] In an effort to determine if antrectomy in dogs and rats produces gastrin deficiency, we undertook two animal studies. The effect of antrectomy on gut function was also assessed.

METHOD

Dog Study

We performed antrectomy on four adult mongrel dogs by removing the distal half of the stomach and re-establishing intestinal continuity by attaching the remaining stomach end-to-side to the proximal jejunum. The duodenal stump was closed to exclude the duodenum from the food stream. The dogs were also fitted with a cannula in the second portion of the duodenum so that a biopsy tube could be passed. The animals received subcutaneous fluids for four to five days after surgery and were then gradually placed on a regular diet. A total of six to eight weeks was allowed to elapse before we began the study.

The study was divided into five one-week periods. The first week was a baseline period during which a distal duodenal biopsy was performed on the dogs before feeding each morning and two hours after feeding. A Quinton Multiple Biopsy device* was employed and about four pieces of tissue were obtained each time. The biopsies were immediately frozen in liquid nitrogen and stored at -80°C until disaccharidase activities could be measured by the method of Dahlquist.[26] Serum gastrin levels were determined before and 30 minutes after feeding two to three times before antrectomy and each day for the first four days of week one. The Schwartz-Mann Gastrin Radioimmunoassay kit,* which measures G-17 and larger gastrins, was utilized for all gastrin determinations.

During the second week of the study, the dogs received Carnation® powdered milk formula mixed 30 ml of powder to 60 ml of water via the duodenal cannula. This was given once a day, each morning, just before oral feeding. This

maneuver was designed to stimulate the duodenum which was now outside the normal food stream. Biopsy and serum gastrin determinations as in period one were carried out on the seventh and tenth day of feeding.

The third period, which consisted of the remainder of week three, was a rest period during which dogs were fed normally by the oral route.

On the first two days of the fourth period, the dogs received pentagastrin (a gift from Ayerst Laboratories), 4 µg/kg subcutaneously every hour for 14 hours. Biopsies for disaccharidases and ^{14}C leucine incorporation done *in vitro*[27] were obtained before beginning the pentagastrin and two and seven hours into the injection periods. After the 14-hour period, another biopsy was obtained for disaccharidases only. Serum gastrin levels were determined at 0, +15, +30, +45, +60 minutes, two hours and seven hours into the injections. Biopsies were also done on the third, fourth, fifth and eighth days of this period to follow the effect of exogenous pentagastrin.

During the fifth week, beginning on Tuesday, duodenal feeding was again begun just before feeding and two hours after feeding on the fifth and seventh day of duodenal feeding. Serum gastrin was drawn before and 30 minutes after oral and duodenal feeding.

Rat Study

To study the effects of gastrin deficiency plus starvation, we performed antrectomy on a group of 27 Charles River Sprague-Dawley rats after anesthetizing them with sodium pentabarbital. The antrectomy was performed by transecting the stomach on the lesser curvature just distal to the esophageal gastric junction across to the midpoint of the greater curvature. The proximal stump was closed and the remaining distal stomach removed by cutting it free from the duodenum just distal to the pylorus. The duodenal stump was closed and a side-to-side anastomosis of the duodenum and remaining stomach was performed. As controls, a gastric transection with immediate reanastomosis was performed on 28 rats, a sham operation.

Following surgery, the rats were given subcutaneous injections of half normal saline and 5% glucose for two days. They were then begun on oral feeding of powdered Purina® rat chow.

The animals were pair-fed, utilizing one or two sham rats for each antrectomized rat.

Half of each group of rats was fasted for two days, following which they were killed with a blow to the head. The killing and subsequent analysis of the intestinal tissue was conducted in half the animals two weeks after surgery, and in the remaining animals six weeks after surgery. Thus, there were eight groups: Sham-fasted and antrectomy-fasted groups two weeks after surgery; fed-sham and fed-antrectomy groups two weeks after surgery; fasted-sham and fasted-antrectomy groups six weeks after surgery; fed-sham and fed-antrectomy groups six weeks after surgery.

Pieces of duodenum, jejunum, ileum and colon were incubated *in vitro* with ^{14}C leucine as in the dog study. Pieces of tissue from mid-duodenum and proximal jejunum were frozen and assayed as in the dog study for disaccharidases. Blood for serum gastrin was collected from all animals by cardiac puncture. Gastrin levels were also measured in a group of 10 normal rats (control rats).

STATISTICS

Student's t-test for correlated means was used for the dog experiments. The rat experiment was analyzed using the Student's t-test for independent means.

RESULTS

Dog

Antrectomy with Billroth 2 type anastomosis did not render the dogs gastrin-deficient. Table 1 presents the pre- and post-antrectomy gastrin levels for the four dogs. Throughout the experimental manipulations and pre- and post-feeding, gastrin levels did not appreciably change in

each dog. One animal had lower levels of gastrin after antrectomy, but these levels were about equal to all the other dogs pre- and post-antrectomy.

TABLE 1

SERUM GASTRIN LEVELS pg/ml IN DOGS

Dog Number	3276		3285		3287		3341	
Feeding state	Fast	30 min after feeding	Fast	30 min after feeding	Fast	30 min after feeding	Fast	30 min after feeding
Pre-antrectomy	not available		92	--	--	--	310	340
Post-antrectomy baseline	70	80	72	92	150	140	72	80
After 7 days of Duodenal feeding	72	72	92	94	132	130	68	68
Pre-pentagastrin	84	--	74	--	118	--	94	--
+ 6 hours 1st day of pentagastrin	84	--	84	--	120	--	100	--
Pre-pentagastrin injection 2nd day	60	--	90	--	120	--	100	--
+ 6 hours 2nd day of pentagastrin	--	--	88	--	130	--	74	--
After pentagastrin and 5 days of duodenal feeding	70	70	110	110	132	150	60	64

Disaccharide levels for the four animals are presented (Fig. 1). Two of the animals, 3285 and 3287, had low levels of both lactase and sucrase throughout the experiment. The levels were higher in the other two dogs, but there was no consistent pattern of change in relation to the experimental manipulations. Combining the lactose and sucrose data from all the animals (Fig. 2), there is no significant change from pretreatment values.

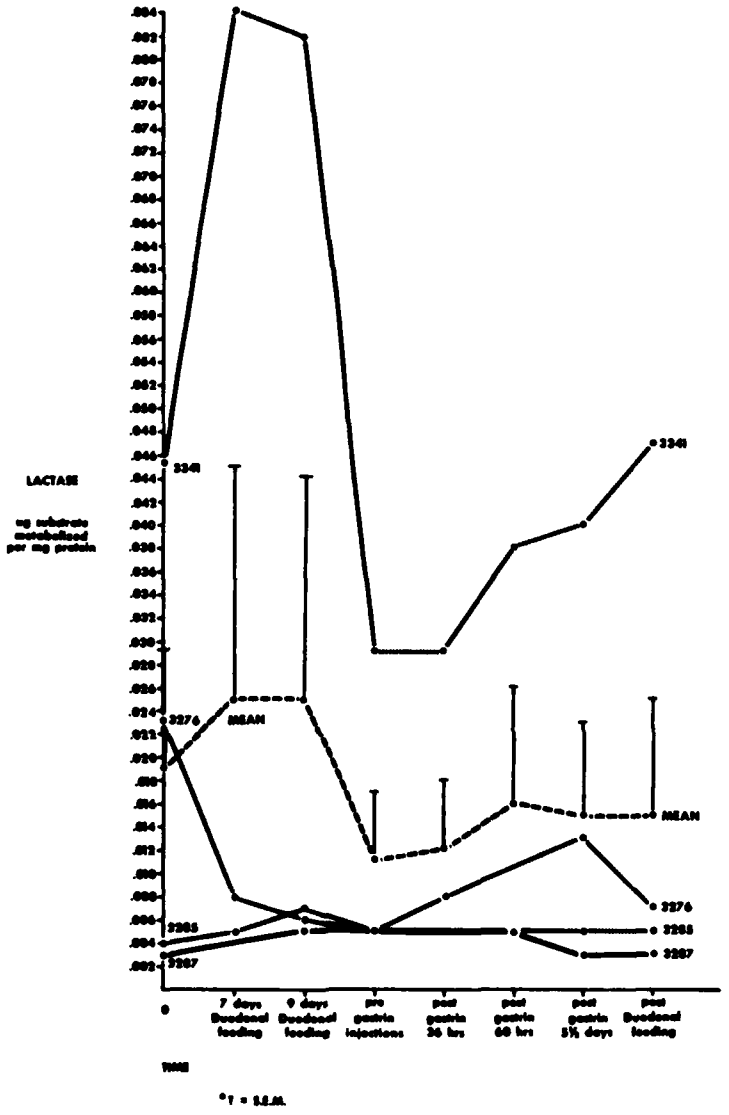

Figure 1. Individual and mean duodenal lactase levels from four antrectomized dogs treated with duodenal feeding and subcutaneous pentagastrin.

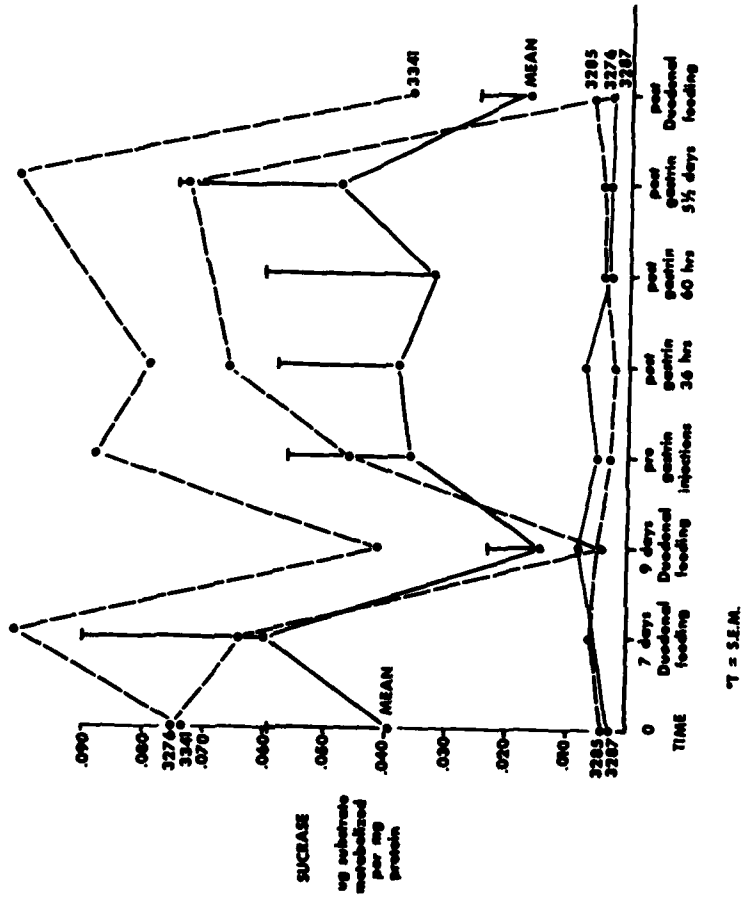

Figure 2. Individual and mean duodenal sucrase levels from four antrectomized dogs treated with duodenal feeding and subcutaneous pentagastrin.

The maltase levels were more consistent among the dogs, as shown by the standard error of the mean (Fig. 3). Here, too, there was no change induced by the experimental manipulations.

Using ^{14}C leucine incorporation as an index of protein synthesis, there appeared to be some stimulation of protein synthesis by gastrin, but the change was not statistically significant. Figure 4 shows the percentage of change in incorporation, which increased during the first day and then returned to baseline. Individual dogs showed an increase after administration of gastrin but increases tended to occur at different times.

Rat

Antrectomy did not render the rats gastrin-deficient. There was no difference in serum gastrins among the normal rats, sham-operated or antrectomized rats in any group except in the two-week postsurgery fed groups, which had a significant increase over the other groups (Fig. 5).

Both lactase and sucrase levels were increased above those of the control animals in all antrectomized groups (Fig. 6). Six weeks after surgery, lactase and sucrase activity began to rise in the control groups and approached levels seen in the antrectomized animals.

Maltase, the most plentiful of the disaccharidases, was the same in all groups except in the six-week antrectomized groups in which jejunal maltase was significantly higher.

The *in vitro* ^{14}C leucine (Fig. 7) incorporation experiment did not show any overall pattern of change. The fasted antrectomized rats had the highest rates of leucine incorporation in the colon for both two- and six-week postsurgery groups. But the levels were not significantly higher than the sham groups. The four tissue areas which did show a significant change were in antrectomized groups of rats. Gastrin levels in the groups did not correlate with increases in ^{14}C leucine incorporation.

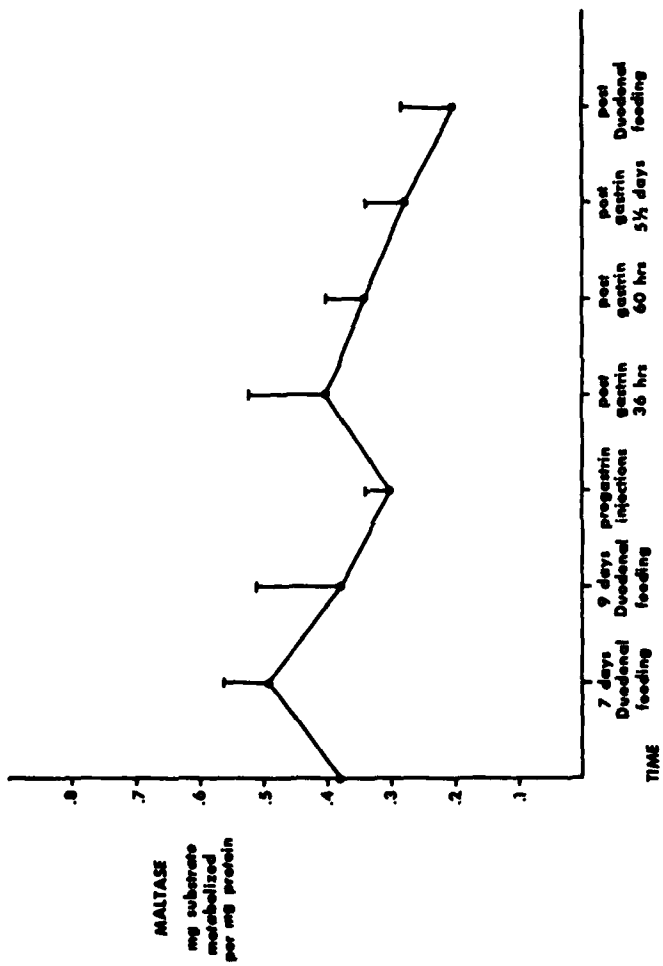

Figure 3. Mean duodenal maltase results from four antrectomized dogs treated with duodenal feeding and subcutaneous pentagastrin.

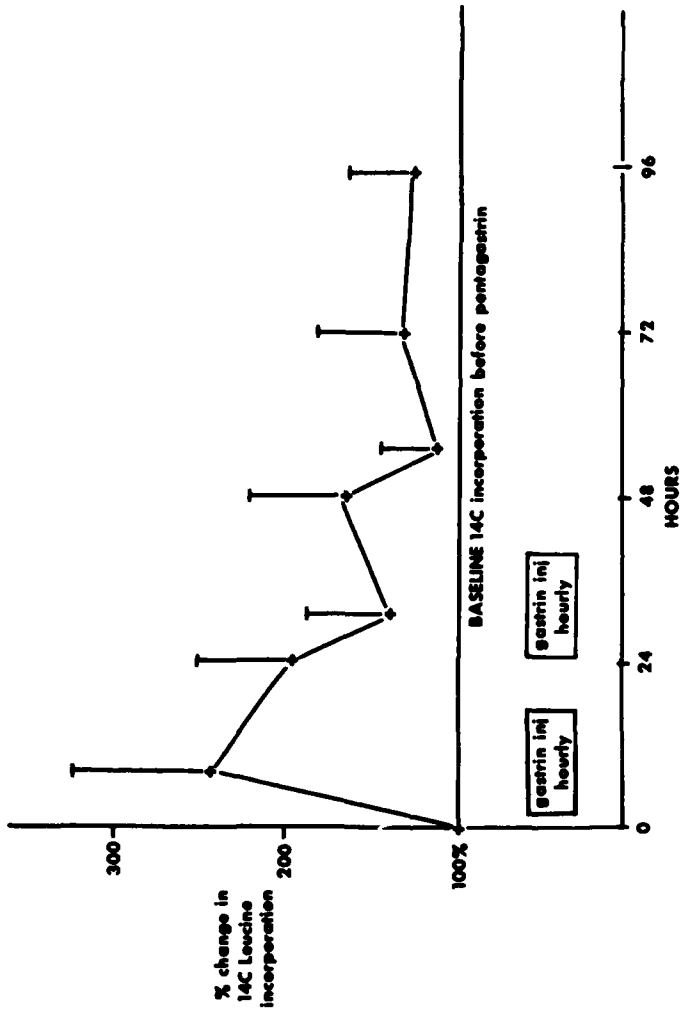

Figure 4. Mean percentage change in *in vitro* ^{14}C leucine incorporation into protein of duodenal biopsies from four antrectomized dogs before, during, and after subcutaneous administration of pentagastrin.

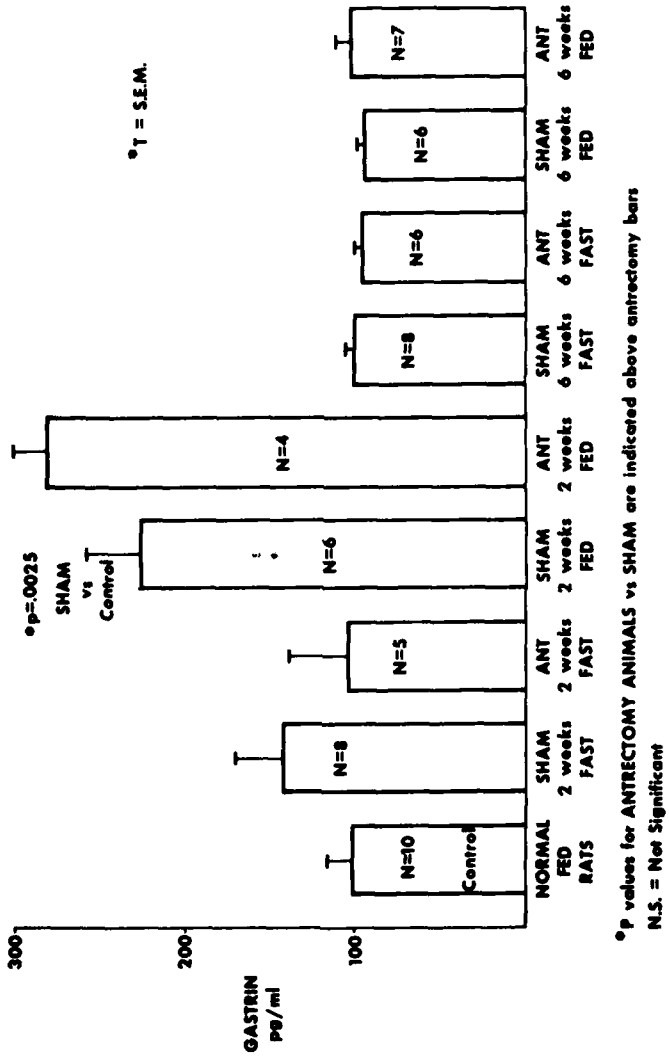

Figure 5. Mean serum gastrins from groups of normal fed rats (control), sham operated rats and antrectomized rats.

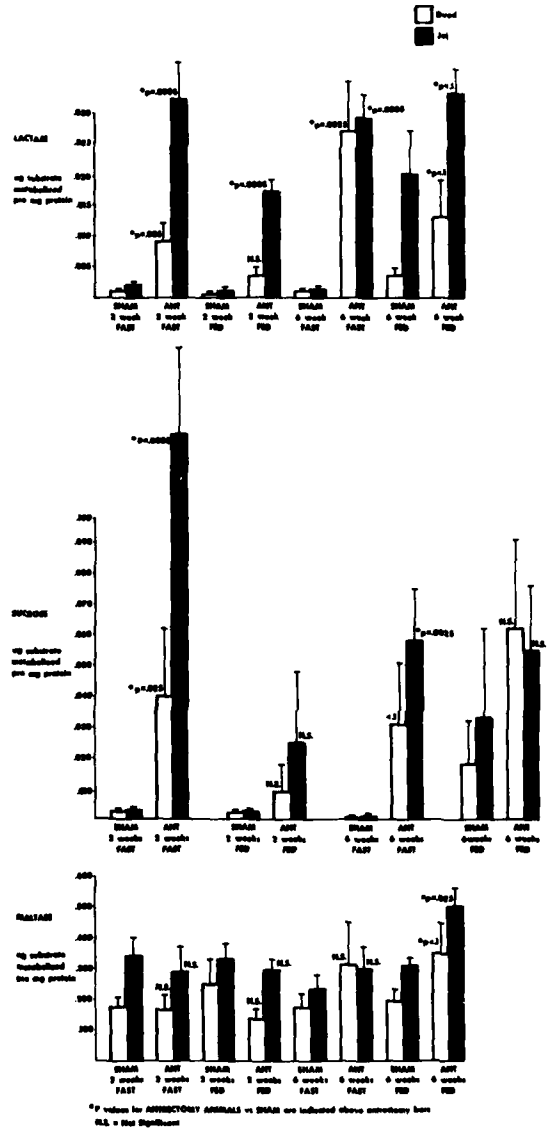

Figure 6. Mean duodenal and jejunal maltose, sucrase and lactase levels from groups of sham operated and antrectomized rats.

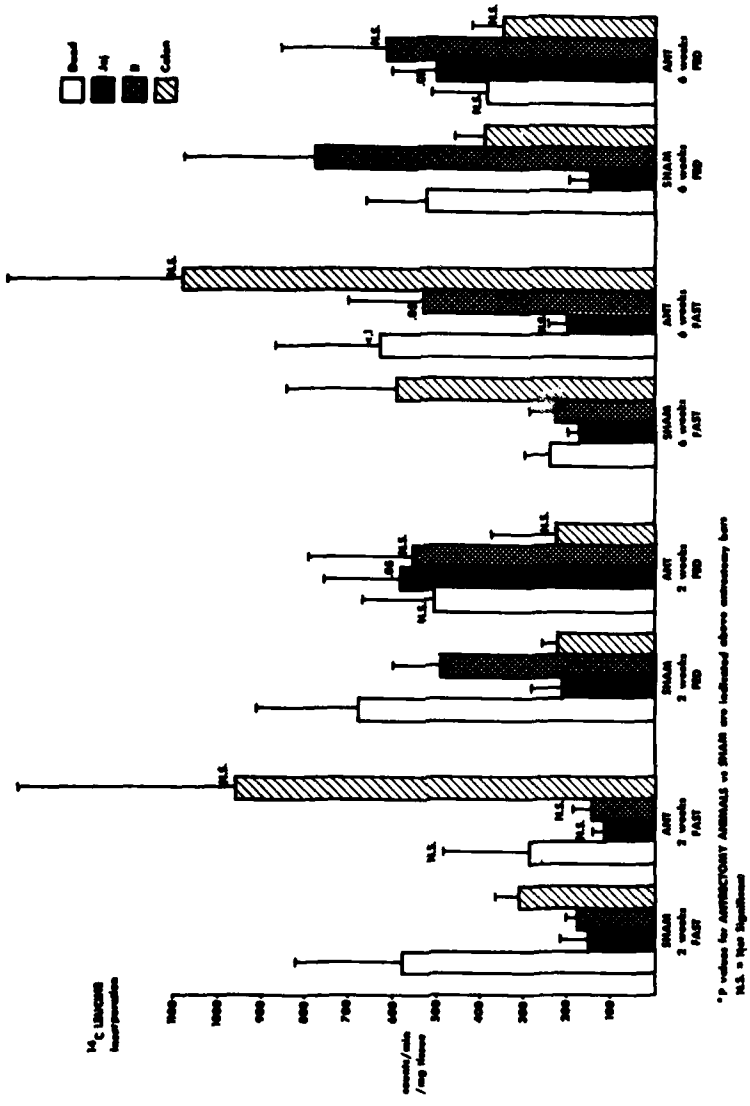

Figure 7. Mean percentage change in *in vitro* ^{14}C leucine incorporation into protein of biopsies from the duodenum, jejunum, ileum, and colon of sham operated and antrectomized rats.

DISCUSSION

The trophic effect of gastrin on the intestine as measured by disaccharidases and ^{14}C leucine incorporation is not supported by our studies. This may be due to failure to render the animals gastrin-deficient. Mak[28] showed that while fasted rats increased ^{3}H thymidine labeling of duodenal and colonic crypt cells in response to a pentagastrin injection of 250 µg/kg, fed rats did not. Presumably this is because of the higher circulating levels of gastrin in fed rats which already had maximally stimulated DNA synthesis. Most rat studies employing pentagastrin injections to assess trophic effects of gastrin used one dose of 250 µg/kg, which Johnson et al[7,8,10,27] found provides peak ^{3}H thymidine incorporation in the rat. Hansen et al[11] used 10 µg/kg/hr for two hours in humans. The dog's hourly dose of 4 µg/kg for 14 hours resulted in a daily dose of 54 µg for two days, which is midway between the usual rat dose and Hansen's human dose. Therefore, it seems unlikely that the lack of effect by pentagastrin is solely due to insufficient dose.

Lichtenberger et al[16] reported that in the rat the rise in disaccharidases during starvation was partially reversed by exogenous gastrin administered during the fasting period. They speculated that the slower turnover of intestinal lining cells caused by starvation led to greater maturation of the cells and higher enzyme levels. Our lactase, but not our maltase results in the fasted rat, coincide with those reported in their study. They measured the disaccharidase activity in the entire gut, whereas in the present study, only a small biopsy from the duodenum and jejunum was measured. Lactase and sucrase are present in much smaller quantities than maltase in both the rat and dog, which may result in their greater sensitivity to induction by environment stimuli. However, lactase in the dog was not increased by feeding lactose. In the rat, the levels of lactase and sucrase appear to be dependent on feeding state but not endogenous gastrin levels. The dogs also were unaffected by exogenous pentagastrin administration.

The intriguing rise in serum gastrin of rats in the two-week fed group raises the question of whether the rise was induced by the surgery or is related to the healing process. By six weeks after surgery, the elevated gastrin

levels were no longer present. The elevated gastrin was induced by both sham and antrectomy procedures but only in the fed group. Several authors have shown that serum gastrin rises after small bowel resection in humans[29] and dogs.[30-34] In addition, Straus[29] reported that in one patient with a partial gastrectomy and a short bowel, there was hypergastrinemia. Two possible explanations are offered by these authors for the high gastrin levels: Either the resected gut was a major area for gastrin degradation, or a gastrin inhibitory factor was removed along with the gut.

A significant rise in gastrin levels following other types of gastric or intestinal surgery has not been reported, nor has the duration of hypergastrinemia following small intestinal resection been completely determined. By 10 days post-resection, elevated gastrin levels are present in dogs.[33] Reports on humans state that ulcers develop in some post-intestinal resection patients,[35-37] so presumably the hypergastrinemia continues for long periods after resection. This is not true for our study. A transient change in the dynamics of stomach-emptying after surgery might be the cause of higher gastrin levels, although it seems unlikely since the effect was present in both sham and antrectomized animals. During the first two weeks after surgery, the stomach is still actively healing and starvation for two days would slow this process, so that elevated gastrin levels may be a marker of active healing. The dog portion of our study did not help clarify this problem because we did not measure gastrin levels in the dogs until at least four weeks after antrectomy. Further research is needed to determine if an elevated gastrin level is inevitably found during healing of gastric wounds.

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GASTRIN IN THE HUMAN SMALL BOWEL

*Barbara Morgan Nylund, MD
*Robert H. Herman, MD, FACP

Gastrin regulates the metabolism and growth of the gastrointestinal tract.[1] Marked hyperplasia of the gastric mucosa has been seen in patients with gastrinoma.[2] The volume of mucosa, the number of parietal cells, and the capacity to secrete acid may be increased as much as six times normal. Hyperplasia of the gastric mucosa can be produced experimentally in rats by large doses of pentagastrin given repeatedly over a period of several weeks,[3] and, conversely, antrectomy causes atrophy of gastric mucosa. The decreased DNA and RNA content of gastric mucosa that follows antrectomy in the rat has been largely reversed by administration of exogenous pentagastrin.[1] In man, antrectomy reduces the maximal secretory capacity of the stomach by about 50%. This decrease can be partly prevented by infusion of pentagastrin given continuously during the first week after antrectomy.[4] The profound atrophy of the stomach, small intestine, and pancreas that occurs during starvation in the rat is associated with marked decreases in serum and antral levels of gastrin and can be prevented by continuous infusion of pentagastrin.[5] Single doses of gastrin stimulate synthesis of DNA, RNA and protein and cause a burst of mitotic activity reaching a peak about 12 hours after the injection. The effect on DNA synthesis has been shown on the pancreas and on the mucosa of the stomach and small intestine of the rat, but effects have not been found in nongastrointestinal tissues.

In the rat, intravenous hyperalimentation was found to decrease intestinal maltase and sucrase.[6] Tissue gastrin fell concomitantly. The disaccharidases were restored to normal control levels by pentagastrin, suggesting that gastrin may control intestinal disaccharidases. Both tissue gastrin and intestinal disaccharidases returned to normal after oral feeding. Increased activity by jejunal

*From Letterman Army Institute of Research, San Francisco, CA.

glycolytic enzymes in response to carbohydrate meals has been demonstrated.[7,8] Food intake influences gastrin and intestinal enzymes, and gastrin has documented trophic effects on the gut of rat. It is hypothesized that gastrin has a generalized trophic effect on the synthesis of protein in the gut. If the trophic effect of gastrin is required for normal protein synthesis, then gastrin deficiency after gastric resection could result in abnormal protein synthesis and subsequent maladaptation of intestinal enzymes, and decreased cell turnover in the gut.

METHODS

The effect of pentagastrin on ^{14}C leucine incorporation into jejunal tissue *in vitro* and on selected jejunal enzyme activities in human volunteers and patients with various gastrointestinal disorders (post vagotomy and pyloroplasty, post vagotomy, antrectomy and post 80% gastrectomy) was studied.

In each subject, maximal gastrin output was determined after a high protein gastrin test meal. Pentagastrin (a gift from Ayerst Laboratories) (ó µg/kg/min) was infused for 16 hours. Peroral proximal jejunal biopsies were obtained before and for three days after infusion. One of the daily jejunal biopsies was incubated in medium 199 solution with ^{14}C leucine for two hours at 37°C. The incubation was terminated by adding trichloroacetic acid to the incubation medium. The samples were then placed in test tubes and plunged into liquid nitrogen and stored at -70°C. The specimens were then analyzed for ^{14}C leucine incorporation into precipitable protein.

Biopsy specimens obtained simultaneously were analyzed for the concentration of disaccharidases and glycolytic enzymes (Tables 1, 2, 3).

TABLE 1

BASELINE SERUM GASTRIN LEVELS ^{14}C LEUCINE INCORPORATION AFTER TEST MEAL, BEFORE PENTAGASTRIN INFUSION

	Serum gastrin % increase 15 minutes after test meal	^{14}C leucine incorporation Day 1	Day 2	Day 3
Control (N=9)	399 ± 17 %	192 ± 31	150±42	105±35
Vagotomy (N=2) and pyloroplasty	333 ± 20	100 ± 50	250±75	302±12
Antrectomy (N=3) Billroth I Anastamosis	250 ± 70	225 ± 50	178 ±10	112±12
80% gastrectomy (N=1) Billroth II Anastamosis	100	401	612	490

TABLE 2

% INCREASE AFTER PENTAGASTRIN INFUSION DISACCHARIDASE ACTIVITIES

		Lactase	Sucrase	Maltase
Control (N=3)	1	167%	175%	150%
	2	233%	102%	178%
	3	123%	97%	228%
Vagotmoy and pyloroplasty	1	0.32	0.30	0.36
	2	0.51	0.52	0.47
	3	--	--	--
Antrectomy with Billroth I	1	1.27	0.96	1.01
	2	1.51	0.98	1.05
	3	1.78	0.87	0.89
Gastrectomy with Billroth II	1	--	--	--
	2	1.06	0.96	0.93
	3	1.44	0.68	0.85

TABLE 3

GLYCOLYTIC ENZYMES CHANGE AFTER PENTAGASTRIN INFUSION

		Pyruvate kinase	FDPase	F-1-Aldolase	FDP Aldolase
Control (N=2)	1	1.0	1.77	1.20	1.09
	2	0.82	0.58	0.73	0.97
	3	0.85	0.67	0.68	0.72
Vagotomy and Pyloroplasty (N=1)	1	0.58	0.46	0.92	0.43
	2	--	--	--	--
	3	0.97	0.53	0.95	0.79
Antrectomy	1	1.51	1.88	1.03	0.88
	2	1.81	2.24	2.45	1.17
	3	1.51	1.29	1.71	1.17
Gastrectomy	1	0.80	0.53	0.75	0.58
	2	0.95	0.62	1.01	1.32
	3	0.93	0.54	0.70	1.02

RESULTS

a. *Gastrin stimulation.* The control subjects showed a maximal gastrin output of 399 ± 17 over basal gastrin output 15 minutes after the high protein test meal. Likewise, the subjects who had had vagotomy and pyloroplasty but who had retained intact antra exhibited maximal gastrin outputs 15 minutes after the test meal of 333 ± 20.

In the antrectomized subjects, there was an increase of serum gastrin of 250 ± 70, but only 62% of control.

The one subject who had both vagotomy and 80% gastrectomy exhibited no change in gastrin output after the gastrin test meal.

b. ^{14}C-*leucine incorporation.* After stimulation with 6 µg/kg/min pentagastrin for 16 hours, all control test groups demonstrated increased ^{14}C-leucine incorporation into protein. Significantly, the one subject in whom no increase in gastrin could be elicited after a test meal, and who had had 80% subtotal gastrectomy, demonstrated the most striking increase in ^{14}C-leucine incorporation: 612% over her own basal level and 319% over the maximal increase in control subjects.

c. *Disaccharidase levels.* The levels of lactase, sucrase and maltase increased in controls after pentagastrin stimulation, but there was a decrease or no significant change in test subjects.

d. *Glycolytic enzyme levels.* Levels of glycolytic enzymes were not markedly affected by pentagastrin stimulations except for fructose diphosphatase which showed a 177% increase after 24 hours. There was no increase in glycolytic enzyme levels in the patient with vagotomy and pyloroplasty, and, indeed, a 50% *decrease* was noted. Whether this is significant can not be determined from the limited data. In the antrectomized subject, there was elicited an increase in all glycolytic enzymes studied, although the increase was minimal for fructose diphosphate aldolase.

In the subject with subtotal gastrectomy, there was no significant change in pyruvic kinase, fructose-1-phosphate aldolase, or fructose diphospho aldolase, and a 50% decrease in fructose diphosphatase.

DISCUSSION AND CONCLUSION

It is not possible to draw conclusions from such a small sample size, but trends can be noted. Pentagastrin seems to stimulate protein synthesis in the normal subject, and, in the gastrin-deficient subject, a marked stimulation in protein synthesis is observed.

The increase of disaccharidase activity after pentagastrin stimulation and the lack of change or decrease in disaccharidase activity in experimental subjects cannot be

explained adequately with so little data. It is likely, however, that gastrin elicits no effect upon disaccharidase activity.

The data for glycolytic enzymes is equivocal but awaits further study. Quite likely, it shall be found that glycolytic enzyme activity, like disaccharidase activity, is unaffected by gastrin, and that the trophic effects of gastrin are mainly those involved in increased cell production and cell size.

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